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5-NITRO-2-(3-PHENYLPROPYLAMINO) BENZOIC ACID, (5-NPB), IS A NON-COMPETITIVE ANTAGONIST AT THE ASCARIS GABA RECEPTOR

L. Colquhoun, L. Holden-Dye* & R.J. Walker, Dept. of Neurophysiology, SOBAPS, University of Southampton, Bassett Crescent East, Southampton, SO9 3TU.

A novel GABA receptor on the somatic muscle cells of the parasitic nematode Ascaris (Holden-Dye et al., 1988) is neither bicuculline nor picrotoxin sensitive though it is activated by GABA-A agonist and the hyperpolarization is due to an increase in chloride conductance.

In this study intracellular recordings were made from Ascaris muscle cells using conventional electrophysiological techniques. Drugs were applied by local perfusion. The GABA response was not blocked by 10 µM TBPS (t-butylbicyclophosphorothionate, n=2) or by 100 µM dieldrin (n=2). We tested analogues of benzoic acid which block chloride conductance in other tissues (Palade & Barchi, 1977; Wangemann et al., 1986). Anthracene-9-carboxylic acid (100 µM) was ineffective (n=2), 4-(4-benzoylanilino)butyric acid was a weak inhibitor (IC_{50} 153 µM, n=3) and 5-NPB was a moderate, non-competitive inhibitor (IC_{50} 69 µM, n=3; Figure 1).

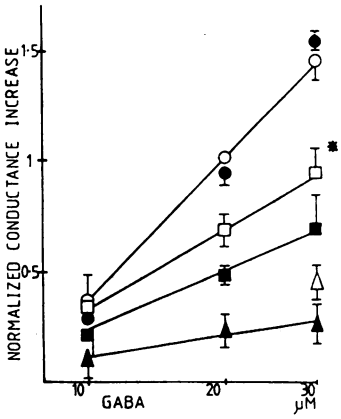

Figure 1. GABA induced conductance increase in the absence (○) and presence of 5-NPB at 10 µM (●), 50 µM (□), 100 µM (■), 500 µM (▲) and after a 5 min. wash (△). In one experiment it was shown that the block was fully reversible after a 90 min. wash. Values are means ± s.e. mean (n=3). * $p < 0.05$ compared to control, paired Student's t-test.

Non-competitive antagonists at the nicotinic receptor e.g. tetraphenylphosphonium (TPP) also recognise the GABA-gated chloride channels (Schwartz & Mindlin, 1988) in rat brain. However, on Ascaris muscle cells 100 µM TPP was without effect though it did block acetylcholine responses on these cells at 10 µM.

Therefore, the GABA-chloride channel in Ascaris is clearly different from the mammalian GABA-A receptor channel. Of the compounds tested so far only avermectin (Holden-Dye et al., 1988) and 5-NPB are antagonists at the Ascaris GABA receptor.

We are grateful to Hoechst for the gift of benzoic acid derivatives and to the SERC for financial support.

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ALLOSTERIC MODULATION BY GLYCINE OF THE NMDA RESPONSE IN GUINEA-PIG MYENTERIC PLEXUS

A.Reggiani*, G.Gaviraghi and R.Ceserani (Introduced by D.G.Trist),
Glaxo-Duncan Research Laboratories, via Fleming 2, 37135 Verona, Italy.

It has been reported that glycine enhances the electrophysiological response of L-glutamate and NMDA in cultured neuronal cells by a positive allosteric modulation of the NMDA receptor through a strychnine insensititve site (Johnson an Ascher, 1987). In this paper we present evidence that such a positive modulatory influence of glycine on the NMDA response is present in guinea-pig myenteric plexus (GPMP) and that the pharmacological characterization of this effect is consistent with the involvement of a strychnine-insensitive glycine site.

Male Dunkin-Hartley albino guinea-pig weighing 300-350 g were used. NMDA-induced ileum contractions were measured in Mg^{++} free Krebs solution according to the method described by Moroni et al. (1986).

The glycine agonist D-serine (10^{-6} to 10^{-4}M) dose-dependently enhanced the contraction induced by a fixed dose of NMDA ($3x10^{-4}$M). At 10^{-4}M a twofold increase of the NMDA response was observed. The presence of strychnine (10^{-5}M) did not affect the D-serine augmentation of the NMDA response. Some other putative agonists at this site were tested and the relevant findings were: glycine was effective in the same range of concentrations (10^{-6} to 10^{-4}M) and with the same intrinsic activity as D-serine; HA 966 (10^{-6} to $3x10^{-4}$M) behaved as a partial agonist at this site having an intrinsic activity about 50% that of D-serine; L-serine was about 100 times less potent than D-serine indicating a stereospecific nature of this site. The putative glycine antagonists 7-Chlorokynurenic acid (7-CL-KYNA), DNQX and Kynurenate were tested as potential inhibitors of this D-serine mediated enhancement and among them 7-CL-KYNA was found to be the more potent. All compounds caused a parallel right shift of the D-serine dose response curve without affecting the maximum suggesting that all of them are competitive antagonists at this site. Based on these experiments the following pA_2 values were estimated: 6.0 for 7-CL-KYNA; 5.3 for DNQX; 4.8 for Kynurenate. An interesting observation was that all compounds non competitively inhibited the NMDA response even in the absence of added D-serine by a glycine-site dependent mechanism. In fact, the simultaneous presence of D-serine (10^{-5}M) fully reversed the inhibitory effect on the NMDA response. To date, it has not been established whether this effect "per se" might be due to the antagonism of endogenous glycine in the tissue, or if it might depend on negative allosterism. If the latter were the case this might imply that in GPMP the glycine site could have both a positive and a negative allosteric influence on the NMDA receptor function.

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GABA-RELATED DRUGS EFFECT ON [^{3}H]-D-ASPARTATE RELEASE

K.S.Rayevsky*, A.V.Prikhozhan, G.I.Kovalev, (introduced by I.C.KILPATRICK), Institute of Pharmacology, USSR Acad.Med.Sci., Moscow, USSR.

It is known, that neurotransmission provided by excitatory amino acids (EAA) can be altered by some of the CNS pathological conditions and can also be involved in the action of a number of neuropsychotropic drugs including neuroleptics and antidepressants of different chemical classes (1). The release of neurotransmitter from the terminals of EAAergic neurones is supposed to be one of the possible sites of action of centrally acting drugs. The aim of the present work was to find out whether some of the anticonvulsants and tranquillizers chemically or functionally related to γ-aminobutyric acid (GABA) affect the K^+-stimulated 3H-D-asparate release (K^+-SDAR) from rat brain cortex synaptosomes which were used in our experiments as an in vitro model of EAA-neurotransmission (presynaptic link).

Tranquillizers Diazepam and Phenazepam (7-bromo-5-(ortho-chlorphenyl)-2,3-dihydroxy-1H-1,4-benzodiazepine-3-one) (10^{-4}M) were found to decrease K^+-SDAR; this effect was antagonized by Ro 15-1788 and enhanced by muscimol via bicuculline- and picrotoxin-sensitive mechanisms. Two other GABA analogues β-phenyl-GABA (Phenybut) and Baclofen (both acting as $GABA_B$ receptor agonists) affect neither K^+-SDAR itself, nor the effect of benzodiazepines. Anticonvulsant Pantogam (pantoyl-GABA) 5×10^{-4}M decreased K^+-SDAR, which was in line with the previous data on acethylcholine release from rat cortex (2), but opposite to the results obtained in the study mentioned, this effect was insensitive to bicuculline, but was reversed by glutamic acid diethyl ester (GDEE) - a well-known antagonist of quisqualate subtype of EAA receptors, which were shown in this laboratory earlier to modulate K^+-SDAR in cortical synaptosomes (1). Another GABA-related anticonvulsant sodium valproate failed to modify K^+-SDAR. It was Piracetam (10^{-6} and 10^{-5}M) but not sodium hydroxybutyrate, that inhibited K^+-SDAR by GDEE-sensitive mechanism. Amnesic substance proline (10^{-4}M) had an opposite effect, which revealed no sensitivity to GDEE, but was antagonized by Piracetam at concentration which failed to modify K^+-SDAR. These findings suggested that some GABA-related drugs are able to modulate EAA neurotransmission via both GABA- and EAAergic presynaptic receptor mechanisms.

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[^{3}H]-GLYCINE AND [^{3}H]-STRYCHNINE BINDING SITES IN HIGHER CENTRES OF GERBIL CNS: AUTORADIOGRAPHIC AND PHARMACOLOGICAL PROFILES

L. Romanelli[1]*, E. Ratti[1] G. Gaviraghi[1], R. Ceserani[1] & N.G. Bowery, Department of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London, U.K. & [1]Biochemical Pharmacology, Glaxo SpA, Via Fleming 2, 37100 Verona, Italy.

Although glycine is well established as an inhibitory neurotransmitter it has also been reported to augment the excitatory actions of N-methyl D-aspartate (NMDA) and glutamate in cultured cortical and diencephalic neurones from mouse embryos (Johnson & Ascher 1987). This latter effect appears to be mediated by glycine receptors which are pharmacologically distinct from those associated with inhibition within the spinal cord and brain stem, in particular strychnine is not an antagonist. The primary purpose of the present study was to determine the location of binding sites for ^{3}H-glycine within the gerbil brain, making comparison with previous data from rat brain (Bristow et al, 1986), and also to establish the pharmacological characteristics of this site. In addition a study of ^{3}H-strychnine binding sites has been made.

Binding of ^{3}H-glycine and ^{3}H-strychnine to 10 um cryostat sections was determined autoradiographically using the in vitro techniques described by Bristow et al (1986) (^{3}H-glycine) and Frostholm & Rotter (1985) (^{3}H-strychnine). Specific binding was determined with glycine (100 uM & 1 mM respectively).

^{3}H-glycine binding sites were distributed unevenly in gerbil brain sections. High concentrations were observed in the hippocampal formation, thalamus, striatum and frontal cortex with much lower levels in the brain stem and molecular layer of the cerebellum. Densitometric analysis of autoradiograms indicated that the highest concentration occurred in CA1 radiatum. The pattern of distribution within the hippocampal formation was the same as observed in rat sections with the exception that the molecular layer of the dentate gyrus exhibited a lower density than any other hippocampal region. By contrast with rat brain 20% of the ^{3}H-glycine bound to gerbil brain sections could be displaced by strychnine. This was not confined to any particular brain region. ^{3}H-strychnine binding sites were observed in many regions of the gerbil brain with the highest density in the brain stem.
Glycine (IC_{50}=0.23$\pm$0.04 uM, mean $\pm$s.e.m.) was the most potent displacer of ^{3}H-glycine binding followed by D-serine (0.63$\pm$0.1), 6,7-dinitro-quinoxaline-2,3-dion (DNQX) (1.06$\pm$0.4), 6,7-dicloro-3-hydroxy-2-amnoxaline-2,3-dion (DCQC) (1.45$\pm$0.6), D-cycloserine (2.87$\pm$0.7), 6-cyano,7-nitro-quinoxaline-2,3-dion (CNQX) (6.03$\pm$1.7), ifenprodil (6.2$\pm$2.4), L-serine (47$\pm$8.2), cycloleucine (160$\pm$90.5), L-cycloserine (407$\pm$159) and taurine (20% displacement at 1 mM) (n=3-4 in all cases). D- & L-serine and D-cycloserine displaced all ^{3}H-glycine binding whereas DNQX, DCQC, CNQX, ifenprodil and cycloleucine displaced only the strychnine-insensitive component. L- & D-serine and taurine (all 1 mM) displaced all specific ^{3}H-strychnine binding.

It is concluded that ^{3}H-glycine binds to strychnine sensitive and insensitive sites in higher centres of gerbil brain. The pharmacological profile of the latter concurs with that associated with the NMDA receptor complex in rat and mouse brain. It is worth noting that the non-NMDA antagonists CNQX, DNQX and DCQC have affinity for the strychnine-insensitive glycine binding site as suggested by Birch et al (1988).

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EFFECT OF 6-CYANO-2,3-DIHYDROXY-7-NITRO-QUINOXALINE (CNQX) ON SPINAL TRANSMISSION FROM PRIMARY AFFERENTS

S.K.Long and R.H.Evans*, DUPHAR B.V., PO Box 2, 1380 AA Weesp, Holland and *Department of Pharmacology, Medical School, Bristol BS8 1TD.

CNQX is a potent antagonist of excitatory amino acid receptors which is, like kynurenate, relatively selective towards non-NMDA types of receptor (Honoré et al, 1988; Fletcher et al, 1988 and Blake et al, 1988). In the immature rat spinal cord the short-latency response of motoneurones evoked from primary afferents is depressed by kynurenate (Ganong et al, 1983), but not by 2-amino-5-phosphonopentanoate (Evans et al, 1982) (AP5), suggesting that it is mediated by an excitatory amino acid acting at non-NMDA receptors. CNQX would be expected to mimic kynurenate at this site. Honoré et al (1988) observed that CNQX, applied to in vivo preparations by the microelectrophoretic method, produced relatively weak depression of spinal neurones excited by cutaneous electrical stimuli. Thus it has been suggested that the transmitter(s) released from low threshold primary afferents may not act at CNQX-sensitive receptors (Foster, 1988).

We have tested the ability of CNQX to depress monosynaptic transmission, recorded from ventral roots following electrical stimulation of dorsal roots (DR-VRP), in the mature in vitro rat spinal cord preparation (Long et al, 1988). In three preparations, at 25°C, stable population spikes of <2 msec latency (amplitude 8.5 mV ±1.0 s.e. mean) were produced by supramaximal electrical stimulation of dorsal roots (0.05 - 0.2 msec pulses). In two of the preparations application of CNQX (10 μM) for 5 min dissolved in the bathing medium reversibly abolished these responses. In the third preparation 2 μM CNQX produced 96% depression within 10 min and 4 μM CNQX abolished the response. Stable recovery of the DR-VRP, following removal of the 4 μM CNQX, occurred after 24 min. The IC_{50} for this effect was estimated, from two preparations, to be <2 μM.

As expected according to previous reports AP5 (100 μM), applied to two of these preparations, had no significant depressant action on the short latency component of the DR-VRP.

These observations show that CNQX is a potent antagonist of the spinal excitatory transmitter(s) released from low threshold primary afferents. Therefore there is no compelling reason to suppose that such transmitters do not act at kainate or quisqualate receptors. The relatively weaker effects seen by Honoré et al (1988) were possibly due to failure of the electrophoretically applied CNQX to reach all of the post-junctional sites in sufficient concentration.

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CIRCADIAN VARIATIONS IN RAT BRAIN KYNURENIC ACID CONCENTRATIONS

J.H. Connick[1], V. Carla[2], F. Moroni[2] and T.W. Stone[1].
[1]Department of Physiology, St George's Hospital Medical School, London, UK., and [2]Department of Pharmacology, University of Florence, Florence, Italy.

The endogenous tryptophan metabolite and excitatory amino acid antagonist, kynurenic acid, has recently been shown to be present in submicromolar concentrations in mammalian brain tissue derived from a wide variety of species including rat and human brain (Carla et al 1988b). During the determination of kynurenic acid levels in the rat brain, it was noted that there were considerable variations in the brain content of kynurenic acid between rats killed at different times throughout the day.
This study was therefore undertaken to explore these changes further.

Male Wistar rats (230-250g) were placed into a carefully controlled temperature (21°C) and humidity (50%) environment on a fixed 12 hour light/dark cycle, six weeks before commencing the experiment. Rats were killed by cervical dislocation followed by decapitation every four hours throughout a 24 hour period. Brains were dissected on ice, weighed and frozen at -80°C until analysis. Kynurenic acid was extracted and analysed as previously described (Carla et al., 1988a).

Figure 1

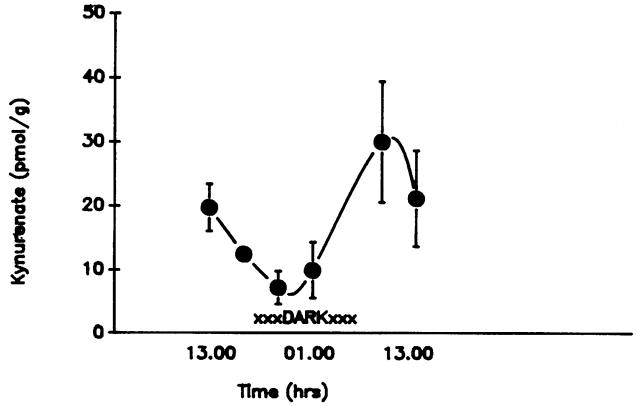

Fig.1 shows the variation in the concentration of kynurenic acid (±s.e.m.) throughout the day. Levels are lowest during the night, rising to a peak shortly after the start of illumination. Levels of kynurenic acid vary over a 24 hour period with a similar brain content at 1pm on the first and second days of the experiment. During the night (9.00pm), kynurenic acid was only 36.3% of that found during the day (1.00pm) ($P<0.05$, $n=4$).

These results demonstrate a circadian variation in rat brain kynurenic acid. The significance of these changes, and any relationship to feeding and activity are currently under investigation.

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PHARMACOLOGY OF QUISQUALATE-INDUCTION OF DEPOLARISING RESPONSE TO L-AMINO-4-PHOSPHONOBUTYRATE (L-AP4) IN RAT CORTICAL WEDGES

D. Lodge and S. Zeman, Department of Veterinary Basic Sciences, Royal Veterinary College, London NW1 OTU.

DL- and L-AP4 depolarise hippocampal and neocortical neurones only after prior exposure of the tissue to quisqualate (Robinson et al., 1986; Harris et al., 1987; Sheardown, 1988; Whittemore et al., 1988) although the mechanism of this action is as yet undetermined. We have investigated the pharmacology of the induction process and of the sensitised L-AP4 site on rat neocortical wedge preparations (Harrison and Simmonds, 1985).

On wedges, treated only with N-methyl-D-aspartate (NMDA;40uM), kainate (10uM) or L-glutamate (4mM), DL-AP4 (10-200uM) had no discernible effect. Following superfusion with a single dose of quisqualate (40uM; 2-3 min), DL-AP4 (100uM) produced a similar sized depolarisation which could be repeated for several hours. The depolarisation increased in amplitude with doses of DL-AP4 up to 500uM and could be larger than that of quisqualate. Depolarisations induced by DL-AP4 100 or 500uM were related to the priming dose of quisqualate (10-80uM). The effect of quisqualate was not mimicked by 4-methylhomoibotenate nor by the isoxazole, AMPA, two "quisqualate receptor" agonists (Krogsgaard Larsen et al., 1980). L-AP4, D-AP4 and DL-AP3 were without apparent effect on cortical wedges before exposure to quisqualate and of these only L-AP4 gave depolarisations after quisqualate. Quisqualate did not increase responses to NMDA, kainate or L-glutamate. The quinoxalinedione, CNQX (1-5uM), and kynurenate (500uM-2mM) reversibly reduced or abolished the sensitised response to L-AP4.

To investigate the nature of the receptors involved in the sensitising action of quisqualate, naive slices were exposed to quisqualate (40uM) during superfusion with known amino acid receptor antagonists, L-AP4 was then tested 1hr after wash out of the antagonist and the quisqualate/L-AP4 protocol re-tested subsequently in drug-free medium. CNQX (2-10uM) appeared to attenuate the induction of L-AP4 responses by quisqualate but the slow reversibility of quisqualate antagonism by CNQX makes interpretation of this data difficult. Kynurenate (500uM-2mM), but not D-AP5 (20uM), also attenuated this effect.

These results suggest that the sensitised L-AP4 receptor has a similar pharmacology to the quisqualate/AMPA receptor but that the receptor inducing the L-AP4 response is not of this type. They also seem incompatible with the concept that depolarisation by L-AP4 is due to release of previously uptaken quisqualate. The possibility of the involvement of second messengers in the described phenomenon is being investigated.

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FAILURE OF TRICYCLIC ANTIDEPRESSANTS AND ZINC TO PRODUCE SELECTIVE ANTAGONISM OF NMDA IN RAT BRAIN *IN VITRO* AND IN *VIVO*

M.G. Jones*, Li Yuliang, D. Lodge and J.A. Millar, Department of Veterinary Basic Sciences, Royal Veterinary College, London NW1 OTU.

It has been recently reported (Peters et al, 1987; Reynolds and Miller, 1988; Westbrook and Mayer, 1987) that zinc cations and some tricyclic antidepressants induce a N-methyl-D-aspartate (NMDA) antagonism by a mechanism separate from that of more classical NMDA antagonists such as magnesium, D-2-amino-5-phosphonovalerate D-AP5 and phencyclidine. We have examined the interactions of tricyclic antidepressants and zinc on excitatory amino acid-induced responses of wedge-shaped slice preparations of rat cerebral cortex and using the technique of microelectrophoresis on single brain stem neurones in pentobarbitone-anaesthetised rats.

Perfusion of rat cortical wedges for up to 2 hours with 10-100uM zinc, desmethylimipramine, nortryptylline and cyproheptadine failed to show any selective antagonism of responses to 40uM NMDA relative to 40uM quisqualate. Doses of the tricyclics greater than this produced depolarisation in their own right and responses to both NMDA and quisqualate were then reduced or abolished. After exposure to zinc, and to a lesser extent after the tricyclics, responses to the amino acids slowly decayed in the wash period. On the other hand 100uM procyclidine, but not 100uM mecamylamine, did give a selective reduction of the response to NMDA.

In electrophoresis experiments *in vivo*, desmethylimipramine (6 cells), mecamylamine (13 cells) and procylidine (17 cells) all reduced action potential amplitude. Lower doses were generally without effect on amino acid responses. With procyclidine and mecamylamine, however, there was some evidence for selective antagonism of NMDA. On 5 of 17 brainstem neurones, procylidine (5-40nA; 25mM in 175mM NaCl) reduced NMDA responses by 72±22% and those to quisqualate by 13±9%. On 7 of 13 neurones, mecamylamine (15-60nA; 25mM in 175mM NaCl) reduced NMDA responses by 53±15% and caused an overall increase of 2±17% in responses to quisqualate.

These results illustrate that, in more intact preparations, the non-specific effects of these compounds on neuronal excitability prevent demonstration of any selective NMDA antagonism.

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TOPOGRAPHY OF THE GLYCINE SITE OF THE NMDA RECEPTOR: PREDICTORS FROM CONFORMATIONAL AND BINDING ANALYSES

D.T. Manallack*, M.G. Wong+, R.D. O'Shea and P.M. Beart, Clinical Pharmacology, University of Melbourne, Heidelberg 3084 and +Victorian College of Pharmacy, Parkville 3052, Australia.

The NMDA receptor-ionophore complex possesses binding sites for phencyclidine-like drugs, competitive NMDA ligands and magnesium ions. Recent electrophysiological and radioligand binding studies have shown that glycine modulates the actions of NMDA via a strychnine-insensitive site in the NMDA receptor-ionophore complex (Bristow et al., 1986; Johnson and Ascher, 1988). Here, the glycine binding site has been investigated using molecular graphic and radioligand binding techniques.

Glycine binding was performed in 5 mM Tris-HEPES containing 1 mM $MgCl_2$ and employed washed synaptic membranes from the cerebral cortices of male Sprague Dawley rats and an incubation at 0-4°C with 10-20 nM [^{3}H]glycine and various drugs. Incubations were terminated at 60 min by centrifugation. Non-specific binding was defined with 200 µM glycine. [^{3}H]Glycine binding was saturable and to a single population of sites: dissociation constant 0.12 µM. Amongst other ligands the rank order of potency was D-serine > D-alanine > 7-chlorokynurenate > D-cycloserine > HA-966 > L-alanine > L-serine > kynurenate.

Computerized molecular modelling was employed to locate low energy conformations, calculate electrostatic potentials, perform energy minimizations and superimpose various molecules active at the glycine binding site. 7-Chlorokynurenate, a glycine antagonist (Kemp et al., 1988), served as the template using the nitrogen atom, a receptor point placed 2.8Å trigonally from the nitrogen atom, atom C2 and the carbonyl oxygen as guide points for superimpositions. A planar binding site was hypothesized, characterized by regions of steric bulk, a lipophilic pocket, an electronegative domain and a domain accepting zwitterions. Additionally, a site accepting the methyl and methylhydroxy groups of D-alanine and D-serine, respectively, was postulated.

Our conformational and radioligand binding studies allow novel insights into the quantitative structure-activity relationships of the glycine site. Additionally, our receptor model predicts actions of quinoxaline-diones as glycine ligands and allows the design of novel ligands.

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5-CARBOXAMIDOTRYPTAMINE MODULATION OF POTASSIUM-STIMULATED [^{3}H]-DOPAMINE RELEASE IN THE RAT NUCLEUS ACCUMBENS

J.M. Barnes*, N.M. Barnes, B. Costall and R.J. Naylor, Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP

A functional relationship between cerebral 5-hydroxytryptamine (5-HT) and dopamine (DA) systems has been identified in biochemical and behavioural studies (Soubrie et al. 1984). In the present studies we investigate the influence of the 5-HT agonist, 5-carboxamidotryptamine (5-CT), on potassium-stimulated [^{3}H]DA release from the rat nucleus accumbens.

The nucleus accumbens of female Hooded-Lister rats (Bradford bred) was removed and cross-chopped at 90° to produce slices of tissue 0.35mm x 0.35mm. The tissue was suspended in normal Krebs solution (mM: NaCl 118.0, KCl 4.75, KH_2PO_4 1.2, $MgSO_4.7H_2O$ 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.0), continuously gassed with 95% O_2, 5% CO_2, and was initially depolarised in a buffer containing 39.7mM KCl prior to incubation in Krebs buffer containing 0.11μM [^{3}H]DA (specific activity 49.9 Ci mM^{-1}) for 40 min. The tissue was washed in Krebs buffer and loaded into Swinnex chambers and superfused with Krebs buffer at a rate of 0.5ml min^{-1}. After a 30 min washout period, 4 min samples of perfusate were collected. At 12 min (S_1) and 48 min (S_2) the slices were depolarised for 4 min in a 20.0mM KCl buffer.

In the absence of any drug the S_2/S_1 ratio was 0.94±0.03 (n = 10). 5-CT (10^{-8}-10^{-5}M) caused a concentration-dependent inhibition of potassium-stimulated [^{3}H]DA release, with the higher concentrations of 5-CT inhibiting some 60% of [^{3}H]DA release. The inclusion of methysergide (10^{-7}-10^{-5}M) in the media antagonised the inhibitory effects of 2.0μM 5-CT in a concentration-dependent manner with values returning to control levels. GR38032F, zacopride, ritanserin, phentolamine, propranolol, atropine, hexamethonium, mepyramine, fluphenazine, ranitidine (1.0μM) and spiperone (0.5μM) superfused alone, failed to modify the potassium-induced release of [^{3}H]DA. Similarly all the above agents failed to antagonise the reduction in [^{3}H]DA release caused by 5-CT (2.0μM). Cyanopindolol, however, at 10nM was able to antagonise the 5-CT response on potassium stimulated [^{3}H]DA release.

Blockade of the 5-CT response by methysergide, but not by ritanserin, GR38032F or zacopride indicates that the ability of 5-CT to inhibit potassium-stimulated [^{3}H]DA release from rat nucleus accumbens tissue may be mediated via a $5\text{-HT}_{1\text{-like}}$ receptor (Bradley et al. 1986) and the antagonism of the 5-CT response by cyanopindolol further indicates that the receptor subtype may be $5\text{-HT}_{1B\text{-like}}$ (Hoyer et al. 1985).

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VENTROMEDIAL GLOBUS PALLIDUS NEURONES REGULATE DOPAMINE FUNCTION IN THE PREFRONTAL CORTEX OF THE RAT VIA THE MEDIODORSAL THALAMUS

Martin W. Jones*, Department of Pharmacology, Medical School, University Walk, University of Bristol, Bristol BS8 1TD. (Introduced by I.C. Kilpatrick)

Midbrain dopamine (DA) neurones innervate *inter alia* the medial bank of the prefrontal cortex (FCx) and the agranular insular cortex (AgCx). These DA terminal regions also receive a non-DA containing input from thalamic mediodorsal (MD) neurones such that lateral parts (MD_L) project to FCx and central parts (MD_C) innervate AgCx. Some functional interaction between these thalamocortical pathways and the DA-containing cortical inputs may exist since we have previously shown that DA utilisation in FCx is enhanced following cell-selective activation of MD_L efferent neurones (Jones et al, 1987). Recent anatomical evidence has shown that cells in rostral ventromedial globus pallidus (GP) not only project directly to FCx but also innervate rostral MD_L and caudal MD_C. In addition, GP cells may influence MD neurones indirectly via the rostral thalamic reticular nucleus (TRN) since GP projects to TRN and dorsal portions of TRN innervate MD_L and more ventral parts project to MD_C. Thus, the present experiments set out to determine the response of cortical DA terminal regions to cell-selective chemical activation of GP. In order to assess whether the cortical responses to this treatment are mediated by (i) a direct corticopetal route or (ii) a route via MD, the experiments were repeated in animals with a 1 week-old lesion of MD_L.

Unilateral stereotaxic injections of the chemical excitants quisqualic acid (QUIS; 1pmol/0.2µl i.e. 5µM) or sodium ibotenate (IBO; 126pmol/0.2µl i.e. 63mM) were aimed at GP of male Porton rats (150-180g) under halothane anaesthesia (1.5% in O_2). Immediately after infusion the animals were killed and DA and its two major metabolites, 3,4-dihydroxyphenylacetic acid (DOPAC) and 4-hydroxy-3-methoxyphenylacetic acid (homovanillic acid, HVA) were determined in FCx and AgCx using HPLC with coulometric detection (Kilpatrick et al, 1986). Lesions of MD_L were produced by allowing 1 week recovery following unilateral infusion into MD_L of *N*-methyl-D-aspartic acid (4nmol/0.2µl i.e. 20mM).

Infusion of QUIS into GP had no immediate effect on DA utilisation (DOPAC:DA and HVA:DA ratios) in either FCx or AgCx. In contrast, infusion of IBO produced an immediate bilaterally symmetrical increase in both DOPAC:DA (ipsilateral: +27%, $p<0.05$; contralateral: +31%, $p<0.01$) and HVA:DA (ipsilateral: +39%, $p<0.02$; contralateral: +36%, $p<0.02$) ratios of FCx. There was also a marked trend towards a bilateral increase of DA utilisation in AgCx. When infusion of IBO into GP was repeated in animals with a 1 week-old MD_L lesion the increased DA utilisation in FCx was now abolished, although the trend towards increased DA utilisation in AgCx was still observed. Since MD_L projects to FCx but not AgCx and since we have previously shown that MD_L lesions **alone** have no effect on DA utilisation in either cortical area (Jones et al, 1987), the present results suggest that the changes in DA utilisation of FCx are mediated via MD_L. Several studies have shown the importance of the thalamus in regulating DA function in various brain regions (Chéramy et al, 1983; Kilpatrick and Phillipson, 1986) and this has now been extended in the present study to include GP, a region which sends incoming afferents both directly and indirectly to the thalamus. (Supported by the Wellcome Trust).

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A COMPARISON OF THE ACUTE AND CHRONIC ACTIONS OF RACLOPRIDE ON CORTICAL, STRIATAL AND AMYGDALOID DOPAMINE (DA) METABOLISM

Eva C. Essig* and Ian C. Kilpatrick, Department of Pharmacology, School of Medical Sciences, University Walk, Bristol, BS8 1TD

Raclopride is an atypical antipsychotic drug and a selective DA-D2 receptor antagonist (Köhler *et al.*, 1985). We have therefore examined its acute and chronic influence on DA metabolism in three brain regions: the amygdala (AM), the medial prefrontal cortex (MPFCX) and the caudate-putamen (CP).

Raclopride (5µmol/kg i.p.) was given either once (acute) or once daily for ten days (chronic) to male Porton rats weighing 230-250 g. Two hours after the final injection, animals were killed and the right AM, MPFCX and CP rapidly dissected. Tissue DA and metabolite (DOPAC and HVA) contents were analysed using an HPLC method (Kilpatrick *et al.*, 1986). Where appropriate, statistical analysis was performed using Student's t-test and the results are illustrated in Table 1:

Table 1 Regional DA Metabolism following Acute and Chronic Raclopride

	AMYGDALA		MEDIAL CORTEX		CAUDATE-PUTAMEN	
	DOPAC	HVA	DOPAC	HVA	DOPAC	HVA
V_A:	40.4 ± 2.6	23.8 ± 1.1	33.3 ± 2.0	38.7 ± 3.0	1680 ± 109	1050 ± 63
A :	+ 61%	+ 74%	+ 66%	+ 308%	+ 224%	+ 378%
V_C:	36.5 ± 2.0	19.6 ± 3.7	35.2 ± 6.5	46.1 ± 5.9	1776 ± 87	742 ± 64
C :	+ 40%	+ 93%	+ 2%*	+ 112%*	+ 139%	+ 278%

V_A and V_C: Acute and Chronic vehicle groups, respectively (pg/mg wet wt ± S.E.M.)
A and C : Acute and Chronic raclopride treatment groups.
* $P<0.01$ when compared with the acute group. (N = 6-8 per group)

The HVA data indicate that AM (innervated by both A9 and A10 DA neurones) is the least and CP (which primarily receives A9 DA inputs) the most responsive area to an **acute** dose of raclopride. However, DA metabolite increases were maintained in the AM and to a lesser extent also in the CP upon **chronic** raclopride treatment. The failure of repeated doses of a neuroleptic to elevate brain DA metabolism to the same degree as an acute dose is a form of tolerance. At the dose used here, chronic raclopride induced marked tolerance in the MPFCX, an area whose DA input derives almost exclusively from A10 DA neurones. This seems surprising in view of the fact that these DA neurones lack synthesis- and impulse-regulating auto-receptors (Chiodo *et al.*, 1984) and typically exhibit less marked neuroleptic-induced increases in DA turnover than other brain areas (Scatton, 1977). By contrast, neither CP nor particularly AM showed marked neurochemical tolerance.

Thus, of those areas studied, raclopride appears to have a selective chronic action on mesocortical DA neurones. The ability of raclopride to maintain an enhanced DA metabolism in A9-innervated areas when administered repeatedly could explain the relative lack of extrapyramidal side-effects seen in the clinic.

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CENTRAL D2 RECEPTORS MEDIATE THE EXCITATORY EFFECTS OF DOPAMINERGIC AGENTS ON THE RAT URINARY BLADDER

G. Iliceto*[1], P. Jenner[2], C.D. Marsden[3] & J.D. Stephenson[4], Departments of [2]Neurology and [4]Pharmacology, Institute of Psychiatry, London SE5 8AF, [3]Department of Neurology, The National Hospital, London and [1]Clinica Neurologica dell'Università degli Studi di Bari, Italy.

A complex dopaminergic influence on bladder function is suggested by clinical findings that bladder hyperreflexia frequently accompanies Parkinson's disease and that paradoxically, the condition is worsened by therapy with dopaminergic agonists (Fitzmaurice et al., 1985). In animals, bladder activity was enhanced by administration of L-DOPA (Sillén et al., 1980) but was suppressed by electrical stimulation of brain regions functionally related to the extra-pyramidal system (Lewin et al., 1967). The excitatory effect of dopamine appears to be the dominant effect and is thought to be mediated by the pontine region of the brain stem (Sillén et al. 1967). The present study attempts to characterize, in rats, the receptors mediating this effect of dopamine.

Wistar rats were anaesthetized (urethane 1.25 mg/kg) and the urethra ligated. A catheter was introduced through the bladder dome and intravesical pressure monitored at constant volume. Drugs were injected intravenously (via a femoral vein), into both striata (in 0.5µl via implanted guide cannulae), or via a catheter introduced through the atlanto-occipital membrane and directed either rostrally, to lie within the IVth ventricle (2.5µl plus 7.5µl saline) or caudally, in the vicinity of the lumbar enlargement (10µl plus 10µl of saline).

Intravenous injections of the selective D_2 agonist, quinpirole (0.03-3 mg/kg) produced repeated co-ordinated rises in intravesical pressure which persisted for at least 90 min and which were indistinguishable from those produced by the non-selective agonist, apomorphine. The effects of both quinpirole and apomorphine (0.5 mg/kg) were antagonised by cis-flupenthixol (5 mg/kg) and by sulpiride (40 mg/kg) but not by domperidone (1 mg/kg), a D_2 antagonist which does not cross the blood brain barrier. Cis-flupenthixol (12.5-25 µg) given into the IVth ventricle was also effective against systemically administered quinpirole. The effects of quinpirole and apomorphine could be mimicked by injections of smaller amounts (0.1-10 µg and 10 µg, respectively) into the IVth ventricle but not after injection of similar or of larger amounts into the striata or lumbar spinal cord. The D_1 agonist, SKF 38393 (1-6 mg/kg i.v. or 10 µg i.c.v.) was without effect.

The results demonstrate that the excitant effects of dopaminergic agents on urinary bladder activity are mediated by D_2 receptors located in the region of the IVth ventricle. The selective D_1 receptor agonist, SKF38393 did not alter bladder activity when given alone nor did it modulate the effects of quinpirole.

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INCREASED NIGRAL IRON AND FERRITIN LEVELS IN PROGRESSIVE SUPRANUCLEAR PALSY

D.T. Dexter[1]*, A. Carayon[2], P. Jenner[1], F. Agid[2], Y. Agid[2] and C.D. Marsden[1]
[1]University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5, U.K. [2]Laboratoire de Medicine Experimental, INSERM U289, Hopital de la Salpetriere, Paris Cedex 13, France.

Neuronal loss in the substantia nigra in Parkinson's disease (PD) is associated with a selective increase in total nigral iron levels but a reduction in ferritin content suggesting altered iron handling may occur in PD (Dexter et al, 1988, 1989; Sofic et al, 1988). However, alterations in iron-related parameters may not be specific to Parkinson's disease. For this reason we have looked at the total iron levels and ferritin levels in brains of patients with the neurodegenerative disease affecting midbrain, tegmentum, substantia nigra, subthalamic nucleus and pallidum namely, progressive supranuclear palsy (PSP).

Brain tissue was obtained from 10 patients dying with PSP (mean age 71.0 years ± 2.2) and from 23 control patients (mean age 81.7 years ± 1.3). Average time between death and removal of brain tissue was 14.0 ± 2.0 h for PSP patients and 8.2 ± 1.0 h for control patients. Brain tissue was solubilized and the iron content measured by inductively coupled plasma (ICP) spectroscopy. Brain ferritin levels were measured by a RIA technique with the antibody being raised against human spleen ferritin.

No differences in total iron content of the cerebellum, cortex and caudate nucleus were observed when tissue from PSP and control patients were compared (Table 1). However, a marked increase in total iron levels was observed in the substantia nigra zona compacta and putamen of PSP patients compared to controls. No difference in ferritin levels were observed in the caudate putamen, cortex and cerebellum when control and PSP patients were compared. However, in the substantia nigra zona compacta of PSP patients there was a marked increase in ferritin content compared to controls (Table 1).

Table 1 Total iron and ferritin levels in control and PSP brains

Brain area	Total iron (nmols/g dry weight)		Ferritin (ng/mg wet weight)	
	Control	PSP	Control	PSP
Cerebral cortex	5693 ± 453	5071 ± 420	30 ± 4	38 ± 6
Caudate nucleus	12515 ± 728	13966 ± 803	148 ± 14	150 ± 15
Putamen	14527 ± 629	19772 ± 1429*	186 ± 17	193 ± 27
Substantia nigra zona compacta	14493 ± 698	24744 ± 2160*	163 ± 16	402 ± 59*
Cerebellum	5741 ± 502	5373 ± 715	45 ± 6	44 ± 7

Values represent mean ± SEM * $P < 0.05$ compared to controls (Student's t test)

In PSP as well as in PD iron levels increase in those areas showing degenerative changes. However, in the substantia nigra in PSP the normal physiological response to increased iron, i.e. stimulation of ferritin synthesis, occurs, in contrast to PD. This might suggest a difference in the role played by iron in these diseases.

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β-ADRENOCEPTOR BINDING SITES IN POST-MORTEM HUMAN BRAIN

F. De Paermentier *, S.C. Cheetham, M.R. Crompton and R.W. Horton.
Department of Pharmacology & Clinical Pharmacology, St George's Hospital Medical School, London SW17 0RE.

Although β-adrenoceptors have been well characterised in rat brain (Rainbow et al, 1984), there are few studies in human brain. In this study we have quantitated and characterised β-adrenoceptor binding sites in post-mortem human brain (from 5 male subjects, mean age 49 years, dying by myocardial infarction, mean post-mortem delay 44 h) and compared the properties of two antagonist radioligands, the widely used [^{3}H]dihydroalprenolol ([^{3}H]DHA) and the more hydrophilic ligand [^{3}H]CGP 12177 (Staehlin & Hertel, 1983).

The ability of propranolol (non-selective β-antagonist), CGP 20712A (β_1-selective antagonist) and ICI 118,551 (β_2-selective antagonist) to displace [^{3}H]DHA and [^{3}H]CGP 12177 (radioligand concentrations close to the K_D, specific binding defined with 200 μM (-)isoprenaline) was compared in cortical and cerebellar tissue. Propranolol displaced both radioligands with uniform affinity in cortex (K_D 2.6 - 2.9 nM) and cerebellum (4.4 - 5.9 nM). In cortex, CGP 20712A displaced [^{3}H]DHA with high affinity from 68 ± 2% of sites (K_D 3.5 ± 1.4 nM) and [^{3}H]CGP 12177 from 92 ± 3% (K_D 2.6 ± 0.5 nM) and the remainder of sites with low affinity. In cerebellum, CGP 20712A displaced only 22 ± 9% of [^{3}H]DHA sites and 40 ± 1% of [^{3}H]CGP 12177 sites with high affinity. In cortex, ICI 118,551 displaced [^{3}H]DHA with high affinity from 40 ± 6% of sites (K_D 4.7 ± 1.2 nM) and [^{3}H]CGP 12177 from 20 ± 4% of sites (K_D 1.9 ± 0.6 nM), whereas in cerebellum 89 ± 2% of [^{3}H]DHA sites and 61 ± 3% of [^{3}H]CGP 12177 were displaced with high affinity. The results demonstrate the selectivity of CGP 20712A and ICI 118,551 for β_1- and β_2-adrenoceptors respectively, in agreement with results in rat brain (Beer et al, 1988).

Saturation binding of [^{3}H]DHA (0.125 - 4 nM) and [^{3}H]CGP 12177 (0.03 - 1 nM) were compared. Binding of [^{3}H]CGP 12177 was of higher affinity (K_D range 0.09 - 0.28 nM) than [^{3}H]DHA (K_D range 0.45 - 1.7 nM) and with a consistently lower percentage of non-specific binding. Both ligands labelled a similar number of sites throughout the brain. Higher densities were found in caudate, putamen and nucleus accumbens (100 - 120 fmol/mg protein) than the cortex (50 - 70 fmol/mg protein); with lowest densities in the cerebellum (40 fmol/mg protein). The proportion of β_1-adrenoceptors (defined by 500 nM CGP 20712A, and expressed as a percentage of the total number of β-adrenoceptors) was highest in caudate (80%), putamen (80%) and cortex (60 - 70%) and lowest in hippocampus (40%) and cerebellum (30%).

[^{3}H]DHA and [^{3}H]CGP 12177 labelled similar numbers of β-adrenoceptors throughout the human brain. The higher affinity and lower non-specific binding of [^{3}H]CGP 12177 make this a preferred ligand to [^{3}H]DHA in human brain.

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EFFECT OF REPEATED IDAZOXAN TREATMENT ON RADIAL ARM MAZE PERFORMANCE IN ADULT AND IN AGED RATS

S.L.Dickinson*, B.Gadie and N.R.Woodmansey, Reckitt & Colman Psychopharmacology Unit, The Medical School, University Walk, Bristol, BS8 1TD and Department of Pharmacology, Reckitt & Colman, Dansom Lane, Hull, HU8 7DS.

Acute pre-treatment with the alpha-2 adrenoreceptor antagonist idazoxan has been demonstrated to reduce forgetting of a previously learned maze strategy (Sara and De Vauges 1986). Furthermore, it has been reported to this society (Dickinson et al 1988) that idazoxan given before but not after training improved retention in a step-through passive avoidance test. Similar to the findings in acute studies, repeated treatment with the less selective alpha-2 antagonist yohimbine improved the rate of acquisition of a food rewarded lever pressing task (Huang et al 1987). We have therefore investigated the effect of repeated treatment with idazoxan given either before (to young adult or to aged rats) or after training (to adult rats) in an 8-arm radial maze.

Food was withdrawn from young adult (7 weeks, weighing 260-340g at the start of training) or aged (23 months, 410-700g) Sprague-Dawley rats who were subsequently rationed to 6g-8g of food per 100g body weight per day (given after training). After an initial 15 minute habituation trial, rats were trained over 9-12 trials at intervals of 24h to 36h to explore for food pellets (45mg, 5 per arm) in each of the 8 arms of a radial arm maze. Training commenced by placing the rat in the centre of the maze in a pre-defined direction. Training was terminated when rats had either visited all 8 arms or had failed to retrieve the food reward within 10 minutes. Performance was assessed by recording the number of errors made (ie. repeat visits to arms) and the total time taken to visit each of the arms at least once. Rats which failed to complete the task in the given time were awarded an error score equivalent to random exploration (14 errors). Idazoxan 1 mg/kg, sc. or saline 1ml/kg was given either 30 min. before or 10 min. following training.

Adult rats given either saline or idazoxan, before training, showed significantly improved performance in both time to complete the task and number of errors made over 9 trials (n=10, Kruskal Wallis ANOVA, saline; trial-1 (median) 330s, 14 errors, trial-9 169s, 3 errors, $P<0.001$: idazoxan; trial-1 359s, 14 errors, trial-9 103s, 3 errors, $P<0.0001$). However, day by day comparison of performance between drug and vehicle treated groups (Mann Whitney U-test, 2 tailed) showed that rats given idazoxan made significantly fewer errors during trial 2 (saline 10.5, idazoxan 4.5, $P=0.031$) and trial 6 (saline 4.5, idazoxan 1.0, $P=0.054$). Furthermore, idazoxan treated rats were significantly faster than saline treated rats on trials 2,6,7 and 9 ($P<0.02$). In contrast, there were no day by day differences when idazoxan was given *after* each training trial. The performance of aged rats given saline, before training, did not significantly improve over 12 training trials as measured by either time taken or errors. Those given idazoxan showed a significant improvement in time taken (n=8, Kruskal Wallis, $P<0.05$) and just failed to show an improvement in errors ($P=0.058$). However, the improvement observed over trials was largely due to a significantly impaired performance during the first training trial (saline 320s, idazoxan >600s, $P<0.01$).

The results indicate that repeated treatment with idazoxan, when given before but not after training to young adult rats improves acquisition performance in an 8-arm radial maze. The response is less pronounced in aged rats who appear less able to perform. This suggests that alpha-2 antagonists may possibly affect motivation, awareness or attention during the performance of the task rather than influencing memory or retrieval mechanisms.

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MODIFICATION OF ELECTROCONVULSIVE SHOCK (ECS) HYPOTHERMIA AFTER SEVERAL DIFFERENT ANTIDEPRESSANT TREATMENTS IN MICE

C.H.Gleiter[1,2], K.M.Wozniak[1], M.J.Costello[1], D.J.Nutt[1,3], & J.DeJong[1]. [1]National Institute on Alcoholism and Alcohol Abuse, Bethesda, MD, USA, [2]Human Pharmacology Institute Ciba-Geigy, Tuebingen, FRG and [3]Reckitt & Colman Psychopharmacology Unit, The Medical School, Bristol, UK.

We have investigated the time course of the hypothermic response to ECS in mice after a single ECS, the last of 10 once-daily ECS and after a single ECS given 1 week and 2 weeks after a course of 10 ECS. In the same fashion the effect of a single ECS on body temperature was measured at different time points (24 h after a single dose, 24 h after 14 days treatment, 1 week and 2 weeks after discontinuation of chronic treatment) following treatment with the antidepressants desimipramine [DMI], fluoxetine [FLUOX], amitriptyline [AMI] and pargyline [PARG]; all 10 mg/kg (i.p. in saline) once-daily. Controls received sham treatment either by earclipping or saline injection.

Body temperature of male NIH Swiss mice was measured using a rectal probe after a single ECS (10mA, 1s via earclips, no anaesthesia) following the above treatments at times 0, 15, 30, 45, 60 and 90 min. Hypothermic responses to the single ECS were calculated as area under the curve (AUC), and compared using ANOVA with Neuman-Keuls test.

A single ECS caused marked hypothermia, which was significantly attenuated by chronic ECS. Chronic antidepressant treatment also significantly attenuated this response to ECS 24 h after the last dose, but not at longer intervals.

Table 1. Hypothermic effect of an ECS after various pretreatments: AUC

After ECS-pretreatment:

Single ECS	Chronic ECS	1 week	2 weeks (after chronic ECS)
149 ± 67	38 ± 18*	56 ± 53*	88 ± 55

After antidepressant pretreatment:

	SALINE	DMI	FLUOX	AMI	PARG
single	188 ± 16	235 ± 53	188 ± 63	223 ± 32	203 ± 44
chronic	200 ± 39	82 ± 11**	52 ± 25**	94 ± 28**	87 ± 27**
1 week	174 ± 44	201 ± 32	143 ± 45	163 ± 51	132 ± 37
2 weeks	154 ± 41	137 ± 22	162 ± 40	125 ± 11	165 ± 25

AUC hypothermia in °C x min; n=8-9 group: mean + SD;
* $p < 0.05$, ** $p < 0.001$

Following a course of ECS and antidepressants there was a clear attenuation of the decrease in temperature caused by ECS. This attenuation was strikingly similar to that seen to the $5HT_{1A}$ receptor agonist 8-OH-DPAT (1) and to clonidine, an alpha-2-adrenoceptor agonist (2), after pretreatment with both ECS and antidepressants. This resistance to the hypothermic effect of ECS may reflect common alterations in brain receptor sensitivity or could suggest a resetting of central temperature control mechanisms.

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REGIONAL DIFFERENCES IN NEUROTRANSMITTER LEVELS AND RESPONSES TO REPEATED ELECTROCONVULSIVE SHOCK (ECS) IN RATS

P. Glue, D.J. Nutt*, A. Pert[1] and M. Costello[2]. Reckitt and Colman Psychopharmacology Unit, The Medical School, University Walk, Bristol BS8 1TD, [1]BPB, NIMH, and [2]LCS, NIAAA, Bethesda, MD 20892, USA.

A number of behavioural and receptor binding changes have been noted in rats after chronic ECS treatment, but effects on neurotransmitter release are unknown. We have recently demonstrated regional differences in neurotransmitter responses to a single ECS using microdialysis (Glue et al 1988), and now report the effects of 8 ECS on basal levels and responses of neurotransmitters in 3 brain regions.

Microdialysis of one of three brain regions (frontal cortex, striatum, or nucleus accumbens) was performed on rats during their 1st and 8th ECS treatments. Before the 8th treatment, a microdialysis probe was inserted into the opposite brain region that had been previously dialysed. Artificial CSF was perfused at 1.33 ul/min, and samples collected every 15 mins, 30 mins after probe insertion. After 4 baseline samples were obtained, a generalised tonic/clonic convulsion was produced by ECS (80mA, 10 sec, ear-clip electrodes), and a further 4 samples obtained. Perfusate samples were analysed immediately by HPLC with ESA coulometric detection. Control animals had similar handling, ear clipping and microdialysis.

Basal noradrenaline (NA) and dopamine (DA) levels were stable in all areas prior to ECS or clipping; these are shown in Table 1.

Table 1.

		DA (fmole +/- sem; n=6)		NA (fmole +/- sem; n=6)	
Region	Treatment	1	8	1	8
Striatum	control	41.2 ± 3.5	23.3 ± 1.9***	-- not	--
	ECS	43.8 ± 5.8	75.5 ± 14.8*	-- detected	--
Nucleus Accumbens	control	14.0 ± 2.0	9.1 ± 0.5	22.7 ± 3.8	12.3 ± 1.0*
	ECS	14.8 ± 1.5	17.9 ± 2.3	26.8 ± 3.2	17.9 ± 2.9*
Frontal Cortex	control	4.1 ± 0.4	3.1 ± 0.5	11.0 ± 1.2	8.1 ± 0.4
	ECS	4.9 ± 1.3	4.6 ± 1.4	9.9 ± 1.4	18.3 ± 2.7***

(* $p < 0.05$; *** $p < 0.01$; treatment 1 v 8).

Peak DA and NA responses after ECS 8 are shown in Table 2; there were no changes in control animals after ear clipping.

Table 2.

	Peak change DA (fmole +/- sem)		Peak change NA (fmole +/- sem)	
Region	ECS 1	ECS 8	ECS 1	ECS 8
Striatum	8.3 ± 4.7	11.0 ± 4.6	-- not detected --	
Nucleus Accumbens	-7.1 ± 1.1	-6.4 ± 1.2	5.3 ± 2.0	3.8 ± 3.9
Frontal Cortex	6.4 ± 1.1	5.0 ± 1.8	19.9 ± 2.3	31.7 ± 4.0**

(** $p = 0.02$, treatment 1 v 8).

Table 1 shows that repeated ECS increases basal DA in striatum and basal NA in frontal cortex. Changes in NA in nucleus accumbens are more difficult to interpret due to the significant fall seen in the control group. Table 2 demonstrates that DA release due to ECS is not altered after chronic treatment, although there was a significant increase in NA release in frontal cortex after chronic treatment. Such changes may be relevant to the receptor and behavioural changes produced by ECS.

Glue P et al. (1988) December Meeting; BPS abstract C49.

PENTYLENETETRAZOLE INCREASES ISATIN CONCENTRATION IN RABBIT BRAIN BUT NOT IN LIVER

A. Clow, J. Davidson[§], V. Glover, J.M. Halket, A.S. Milton[§], M. Sandler & P.J. Watkins, Queen Charlotte's and Chelsea Hospital, LONDON W6 0XG, [§]Department of Pharmacology, University of Aberdeen, ABERDEEN Scotland AB9 1AS

It has been reported that administration of the anxiogenic agent, pentylenetetrazole (PTZ), to rats results in increased endogenous monoamine oxidase (MAO) inhibitory activity ("tribulin") in brain, an effect which can be attenuated by benzodiazepine pretreatment (Bhattacharya *et al.*, 1988). Glover *et al.* 1988 identified the main MAO inhibitory component of tribulin in normal human urine as isatin, an indole not previously known to be present endogenously. We report here an investigation of both isatin concentration and MAO inhibitory activity in rabbit brain and liver following PTZ administration.

New Zealand white rabbits (approx. 2 kg) received either saline or a minimal convulsant dose of PTZ (20 mg/kg) i.v. slowly over 30s via the marginal ear vein. After 30 min, the animals were killed by cervical fracture, the brains and livers were removed and immediately placed in dry ice until analysis. Methods for determination of isatin by GCMS, and MAO inhibitory activity have been described previously (Glover et al., 1988).

The results are shown in the following table.

Isatin concentration (ng/g) in rabbit brain and liver 30 min after PTZ (20 mg/kg i.v.) or saline (C), and specific activity (n moles tyramine oxidised/ mg protein/ 30 min) of a rat liver MAO preparation following incubation with extracts from the same rabbit brains and livers (±SEM)

	Isatin				MAO activity			
	n	PTZ	n	C	n	PTZ	n	C
Brain	11	70.8 ± 7.8*	13	48.8 ± 5.1	11	12.7 ± 0.9**	13	16.9 ± 1.3
Liver	11	167.0 ± 30	11	194.0 ± 25	12	18.4 ± 2.9	13	21.1 ± 1.9

*P<0.05, **P<0.02, compared with control values (Student's t-test)

There was a positive correlation between isatin concentration and MAO inhibitory activity in rabbit brains: n = 24, r = 0.61, P<0.01.

These results indicate that isatin is likely to be a component of what was previously known as tribulin, in animal tissues and human urine, and that both isatin concentration and MAO inhibitory activity are raised in rabbit brain but not liver following PTZ administration.

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IS ENDOCARDIAL CONTROL OF MYOCARDIAL CONTRACTION MEDIATED VIA CYCLIC GMP?

A.M. Shah*, M.J. Lewis & A.H. Henderson, Departments of Cardiology and Pharmacology & Therapeutics, University of Wales College of Medicine, Heath Park, Cardiff.

The contractile performance of isolated mammalian heart muscle preparations is characteristically modulated by the endocardium (Brutsaert et al., 1988). We investigated the possible role of cyclic GMP in this endocardial effect. Ferret papillary muscles (n=41) were mounted at l_{max} in organ baths containing Krebs-Ringer solution (1.25mM calcium, pH 7.4, 29°C), attached to a length-tension transducer and stimulated electrically at 0.2Hz (Chappell et al., 1986). Endocardium was selectively removed by a 2-3 second exposure to 0.5% Triton X-100. Myocardial cGMP levels were measured by radioimmunoassay.

Selective removal of endocardium resulted in a characteristic alteration of contractile twitch : the time to peak isometric twitch tension was reduced with only minimal reduction in the rate of tension development and no reduction in maximum unloaded shortening velocity (V_{max}), thereby causing a small reduction in peak isometric twitch tension - Figure 1. In endocardium-intact preparations, 0.1mM 8-bromo cGMP (Shah et al., 1989), 1μM sodium nitroprusside (SNP) and 0.4μM atrial natriuretic peptide (ANP) - a cGMP analogue, and stimulants of soluble and particulate guanylate cyclase respectively - induced changes in contractile behaviour identical to selective endocardial removal. 1μM substance P - which releases endothelium derived relaxing factor (EDRF) from vascular endothelium - also induced similar changes in endocardium-intact preparations. The effects of substance P were blocked by 10μM haemoglobin, an inhibitor of EDRF activity. Myocardial cGMP levels increased to 408, 488 and 227% of baseline values following SNP, ANP and substance P respectively ($p<0.05$ in each case).

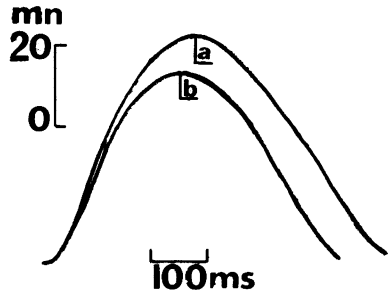

Figure 1. Representative isometric twitches showing effect of selective endocardial removal. a: baseline twitch b: after endocardial removal.

These results show that (a) SNP, ANP and substance P each increase cGMP levels in ferret cardiac muscle, (b) elevation of myocardial cGMP is associated with a characteristic "negative inotropic" effect which reduces the duration of contraction without altering V_{max} - similar to the effect of selective endocardial removal, (c) the effect of substance P may involve release of an "EDRF-like" agent which elevates myocardial cGMP.

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ADRENOCEPTOR-MEDIATED CHANGES IN VENTRICULAR AUTOMATICITY DURING GLOBAL ISCHAEMIA IN PERFUSED RAT VENTRICLES

M.C. Butterfield* & R. Chess-Williams, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

It has been proposed that in the anaesthetised cat, α-adrenoceptor mediated increases in ventricular automaticity are enhanced during myocardial ischaemia and early reperfusion (Sheridan et al., 1980). In the present study we have examined the effects of α- and β-adrenoceptor stimulation on ventricular automaticity in the isolated rat heart. Hearts from male rats were set up under 3g tension and perfused at 37°C by the Langendorff method. The atria were removed, the atrio-ventricular node crushed and the ventricles paced at 4Hz for 20 min. Perfusion pressure was then either a) reduced to $10cmH_2O$ (ischaemic ventricles) or b) maintained at $70cmH_2O$ (controls). 30 minutes later the pacing stimulus was removed and the spontaneous rate of ventricular contractions recorded. Cumulative concentration-response curves to isoprenaline or phenylephrine were then constructed in the presence of metanephrine (10μM).

The resting spontaneous rate of intact perfused hearts (248 ± 9bmin^{-1}) was significantly ($P < 0.001$) reduced after removing the atria (146 ± 10bmin^{-1}). The spontaneous ventricular rate was further reduced to 94 ± 13bmin^{-1} on crushing the atrio-ventricular node ($P < 0.001$).

Table 1: EC_{50} values (with 95% confidence limits) and maximum responses in control and ischaemic ventricles (n = 4).

Agonist	Control	Ischaemic	P
Isoprenaline			
EC_{50} (nM)	3.5 (1.5 - 8.3)	1.8 (0.5 - 6.3)	N.S.
maximum increase (bm^{-1})	86.3 ± 7.5	53.5 ± 8.2	< 0.05
Phenylephrine			
EC_{50} (μM)	28.8 (12.4 - 67.0)	3.0 (0.8 - 18.0)	< 0.05
maximum increase (bmin^{-1})	43.5 ± 15.2	54.8 ± 8.9	N.S.
maximum (% isoprenaline)	48.9 ± 12.4	72.9 ± 5.6	N.S.

After 30 min pacing, the spontaneous ventricular rate in ischaemic tissues (40.9 ± 4.7 bmin^{-1}) was significantly lower ($P < 0.001$) than in controls (73.9 ± 6.2 bmin^{-1}). Isoprenaline produced concentration-dependent increases in rate with similar EC_{50} values in control and ischaemic ventricles although the maximum increase in rate was depressed in ischaemic tissues (Table 1). In contrast phenylephrine produced biphasic concentration-response curves in control tissues, low concentrations (0.1 - 1.0μM) causing a fall in ventricular rate (maximum fall of 13 ± 7.8 bmin^{-1}) while higher concentrations elicited positive chronotrophic responses. In ischaemic tissues, phenylephrine produced only increases in ventricular rate with a significantly lower EC_{50} values than in controls. Also the maximum response to phenylephrine expressed as the absolute increase in rate or as a percentage of the isoprenaline maximum was greater in ischaemic tissues although this was not statistically significant (Table 1).

These results support the hypothesis that α-adrenoceptor mediated increases in ventricular automaticity are enhanced during myocardial ischaemia.

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CHARACTERISATION OF THE TOLERANCE TO THE CARDIOVASCULAR EFFECTS OF SK&F 101468-A IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR)

R.J.Eden, C.A.Harvey, M.Hutchison, D.A.A.Owen*, and S.G.Parker, Smith Kline & French Research Ltd., The Frythe, Welwyn, Herts., AL6 9AR

SK&F 101468-A is a potent, selective agonist at dopamine DA_2-receptors. Substantial falls in blood pressure have been demonstrated following intravenous or oral administration to conscious or anaesthetised SHR (Eden et al 1988a). Further studies have shown that tolerance to the hypotensive effects of SK&F 101468-A occurred following oral b.d. pretreatment (Eden et al 1988b). The present studies were designed to further characterise this phenomenon.
SHR were pretreated with SK&F 101468-A 40 $mg.kg^{-1}$ p.o. every 12 hours for 4 days and anaesthetised with dial-urethane 12 hours after the last dose. A femoral artery and vein were cannulated for the measurement of blood pressure and drug administration. Blood pressure and heart rate were recorded before, and after i.v. administration of either SK&F 101468-A 0.5 $mg.kg^{-1}$ (SKF) or bethanidine, 5 $mg.kg^{-1}$ (BE). Additionally, following the same pretreatments, SHR were pithed and dose-response curves for noradrenaline-induced increases in blood pressure and heart rate constructed. In a further study, in normotensive rats, using a similar dosing regime plasma concentrations of SK&F 101468-A were measured following i.v. challenge with SK&F 101468-A, 0.5$mg.kg^{-1}$

Table 1. Resting mean blood pressure (mmHg) and peak changes after i.v. administration of SKF or BE in SHR following chronic oral pretreatment with saline, or SK&F 101468-A.

Values are mean ± S.E.M. (mmHg)

Acute doses	SALINE		SK&F 101468-A	
	SKF	BE	SKF	BE
n	6	5	6	5
Resting	178±7	204±6	175±11	190±6
Peak effect	-70±7	-74±5	-30±10*	-70±6

*Significantly different from saline control ($p<.01$)

Tolerance to the peripheral effects of SK&F 101468-A which was not overcome by a ten-fold increase in challenge dose, has been demonstrated in SHR (Eden et al 1988b). Plasma concentrations of SK&F 101468-A, following i.v. dosing with SKF, were not reduced by pretreatment with the compound (11.0 ± 2.0 $\mu mol.l^{-1}$ in SKF pretreated, 6.9 ± 1.4 $\mu mol.l^{-1}$ in saline dosed SHR, n=4, peak concentrations) as was end organ response to noradrenaline (0.1-60 $\mu g.kg^{-1}$). Replacing the SKF i.v. challenge with bethanidine caused a response which was similar in both saline and SK&F 101468-A pretreated animals (Table 1).
These data confirm the development of tolerance to the peripheral effects of SK&F 101468-A. This phenomenon does not appear to be due to a change in kinetics, in end-organ responsiveness, or a change in non-sympathetic tone and would be consistent with down-regulation of DA_2 receptors.

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INCREASED β2 ADRENOCEPTOR COUPLING TO INOTROPIC EFFECTS IN HUMAN ATRIAL MYOCARDIUM DUE TO ATENOLOL TREATMENT

Hall J.A. [1]Kaumann A.J. Brown M.J., Clinical Pharmacology Unit, Addenbrooke's Hospital, Cambridge, CB2 2QQ [1]SK&F Welwyn, Herts, AL6 9AR

Atrial myocardium from patients treated with the β-1 selective antagonist atenolol is selectively more sensitive to β-2 adrenoreceptor stimulation by adrenaline than tissue from non β-blocker treated patients (Hall et al 1988). This is unlikely to be due to a change in receptor density since β-2 adrenoreceptor density in human atrial myocardium is unchanged by atenolol treatment (Michel et al 1987). Therefore the increased sensitivity to β-2 adrenoreceptor stimulation could be due to either increased affinity of the receptor or increased coupling of the receptor to effector mechanisms. Salbutamol, a β-2 selective partial agonist was used to determine β-2 adrenoreceptor affinity. Salbutamol's stimulant effect is via β-2 adrenoreceptors since it is blocked by β-2 selective ICI118551 50nM and not by β-1 selective CGP20712A 300nM.
The right atrial appendage was removed from patients, receiving either atenolol or no β-blockers, undergoing routine cardiac surgery. Other medications included calcium antagonists and digoxin but these treatments had no significant effect on the responses. Strips of tissue were mounted in an organ bath at 37°C, paced at 0.5 Hz and incubated for 2 hours with phenoxybenzamine 5μM (Gille et al 1985) and CGP20712A 300nM. Dose response curves (DRC) were by cumulative addition of agonist. First a DRC for adrenaline was determined, and the adrenaline washed out. Next a DRC to salbutamol was determined, then in the presence of salbutamol a second DRC to adrenaline was determined. The affinity of salbutamol for β-2 adrenoreceptors was estimated from double inverse plots of equieffective concentrations of salbutamol and adrenaline, Ks (Waud 1969) and from blockade of adrenaline's responses by salbutamol, Kp (Marano and Kaumann 1976). To allow us to correct for desensitisation paired control tissues were used to determine 2 consecutive DRC's for adrenaline.

TABLE 1

	SALBUTAMOL				ADRENALINE
	Ks(μM)	Kp(μM)	pD2(M)	IA	pD2(M)
Atenolol	0.84	1.02	6.58	0.78	7.53
treated	±	±	±	±	±
n=12	0.18	0.29	0.10	0.04	0.10
			p<0.001	p<0.01	p<0.001
non β-bl.	1.19	1.49	5.78	0.24	6.42
treated	±	±	±	±	±
n=10	0.20	0.78	0.25	0.07	0.17

Values are mean±s.e.m.. Comparisons are by Student's t-test. IA is intrinsic activity of salbutamol with reference to isoprenaline 0.2mM used to terminate the experiment.
The affinities of salbutamol for β-2 adrenoreceptors are not statistically significantly different between the 2 groups of patients or between the 2 methods of analysis. The sensitivity of tissues to stimulation with salbutamol, pD2, correlates with the intrinsic activity of salbutamol ($r=0.89, p<0.001$). There is also a close correlation between salbutamol's intrinsic activity and pD2 for adrenaline ($r=0.91, p<0.001$).

Conclusions 1.Tissues from atenolol treated patients are more sensitive to β-2 adrenoreceptor stimulation. 2.The unaltered affinity of the β-2 adrenoreceptor for salbutamol suggests that the increased sensitivity is not due to increased affinity for agonists 3.This increased sensitivity suggests an increased coupling of β-2 adrenoreceptor to biochemical mechanisms leading to enhanced inotropic responses.

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A COMPARISON OF AZ-DF 265 AND TEA ON THE ACTIONS OF CROMAKALIM, DIAZOXIDE AND MINOXIDIL SULPHATE ON RAT AORTA

D.T. Newgreen*, J. Longmore and A.H. Weston. Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester, Manchester M13 9PT.

Comparative studies using cromakalim (Hamilton et al., 1986) have shown that the vasorelaxant drugs diazoxide and minoxidil sulphate have K channel opening actions on the rat aorta and portal vein (Bray et al., 1988; Newgreen et al., 1989). The relaxant actions of all three substances on rat aorta are antagonised by glibenclamide, which produces apparent competitive inhibition of the relaxant action of cromakalim, whilst a non-competitive pattern of inhibition of minoxidil sulphate is observed (Newgreen et al., 1989). Glibenclamide is known to block ATP-dependent K channels in the pancreas (Sturgess et al., 1988), but the nature of the inhibitory action of this agent against K channel openers in blood vessels is unknown. The present study was designed to compare the action of a novel non-sulphonylurea blocker of ATP dependent K channels in the pancreas, AZ-DF 265 (Garrino & Henquin, 1988) with the effects of tetraethylammonium (TEA) on the action of cromakalim, diazoxide and minoxidil sulphate. Endothelium-free strips were prepared from the thoracic aortae of male Sprague Dawley rats (300-500g) and mounted either for isometric recording in MOPS PSS at 37°C, pH 7.4, or on a manifold for ^{86}Rb efflux studies.

AZ-DF 265 (0.3 - 10μM) and TEA (3-10mM) inhibited the ability of cromakalim, diazoxide and minoxidil sulphate to relax a 20mM KCl contraction. The inhibitory effects of AZ-DF 265 and TEA against cromakalim and diazoxide had the characteristics of competitive antagonism whilst their action against minoxidil sulphate appeared to be non-competitive. AZ-DF 265 (0.3 - 10μM) had no effect on resting tone in rat aorta; it did not affect contractile responses to KCl (20mM) or the ability of sodium nitroprusside (SNP) to relax such contractions. TEA (3 - 10mM) had no effect on resting tone but contractions to KCl (20mM) were potentiated and the relaxant action of SNP was slightly inhibited. AZ-DF 265 (10μM) and TEA (10mM) inhibited the increase in ^{86}Rb efflux produced by cromakalim (1 - 10μM) and diazoxide (0.3 - 3mM). AZ-DF 265 (10μM) and TEA (10mM) had no effect on ^{86}Rb efflux evoked by KCl (20mM) in rat aorta.

These results show that AZ-DF 265 and TEA inhibit the action of cromakalim, diazoxide and minoxidil sulphate in a qualitatively similar manner. This suggests that AZ-DF 265 (and glibenclamide - see Newgreen et al., 1989) act to block the K channel(s) involved in the action of the K channel openers. The observation that AZ-DF 265 is an effective inhibitor suggests that as in the pancreas, the sulphonylurea moiety is not a prerequisite for K channel blockade.

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ENDOTHELIN ACTION ON ADRENAL CHROMAFFIN CELLS TO STIMULATE EFFLUX OF NORADRENALINE AND ADRENALINE

M.R. Boarder* and D.B. Marriott, Department of Pharmacology and Therapeutics, University of Leicester, P.O. Box 138, Medical Sciences Building, University Road, Leicester. LE1 9HN.

Endothelin is a highly potent vasoconstrictor peptide derived from cultured aortic endothelial cells (Yanagisawa et al. 1988). Initial reports apparently showed that endothelin acts at least in part via calcium influx through voltage-sensitive calcium channels, although subsequent studies have cast doubt on this Auguet et al. 1988; Miasiro et al. 1988). Here we report the results of preliminary investigations on the effect of endothelin on catecholamine efflux from cultured bovine adrenal chromaffin cells, a widely used model of neuronal function.

Bovine adrenal chromaffin cells were prepared as described previously (Marriott et al. 1988), were purified by differential plating (Waymire et al. 1983) and cultured at 0.5 x 10^6 cells/well in 'Primaria' 24-well plates. Release of endogenous noradrenaline and adrenaline was measured over a 5 min. period by high performance liquid chromatography with electrochemical detection. The calcium channel enhancer BAY-K-8644 was present, where appropriate, during a 10 min preincubation as well as the 5 min incubation.

Table 1 shows results of an experiment comparing efflux elicited by endothelin 1 ('porcine endothelin') with that elicited by other agents. These results are typical of a number of similar experiments in that endothelin elicits a release which is significant but smaller than that of previously investigated agents.

Table 1 Release of noradrenaline and adrenaline

	Control	Endothelin (30 nM)	Prostaglandin E_2 (10 µM)	Potassium (50 mM)
Noradrenaline	5.2 ± 0.46	19.7 ± 0.51	40.0 ± 1.8	184 ± 5.7
Adrenaline	4.1 ± 0.5	10.5 ± 0.7	26.2 ± 1.8	96.6 ± 3.9

Data are pmols/well, mean ± SEM, n = 4.

Dose-response curves for endothelin show an EC_{50} of about 1 nM. The time course of release elicited by 10 nM endothelin shows that stimulation of release is complete by 5 min. When maximal stimulation by endothelin is combined with submaximal stimulation by 20 mM potassium the release elicited is additive, not synergistic as would be expected if endothelin was acting like BAY-K-8644. Combination of maximal endothelin stimulation with 1 µM BAY-K-8644 gave additive increments of release, not synergistic as would be expected if the release by endothelin was caused by a submaximal depolarisation.

These results show that endothelin is able to elicit a transient stimulation of release of catecholamines from cultured chromaffin cells by a mechanism apparently distinct from either the action of calcium channel agonists or small depolarisation.

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REGIONAL VASCULAR RESPONSES TO ENDOTHELIN IN SPONTANEOUSLY HYPERTENSIVE RATS: COMPARISON WITH WISTAR-KYOTO CONTROLS

Christine E. Wright[1]* and John R. Fozard, Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland, [1]Current address, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.

Endothelin induces a complex cardiovascular response in anaesthetized, ganglion-blocked spontaneously hypertensive rats (SHR); both vasoconstriction and vasodilatation are seen with the contribution of each being dependent on the particular vascular bed being monitored (Wright & Fozard, 1988). We have now compared the regional haemodynamic responses to endothelin in SHR with those from normotensive Wistar-Kyoto (WKY) control rats.

Twenty week old SHR (333 ± 7 g; n=16) and WKY rats (380 ± 6 g; n=16) were anaesthetized with Inactin® 120 mg/kg i.p. and prepared for recording mean arterial pressure (BP) and i.v. drug administration (Wright & Fozard, 1988). Pulsed Doppler flowprobes were placed around the left carotid artery (CAR), left renal artery (REN), superior mesenteric artery (MES) and lower abdominal aorta (equated to hindquarter, HQ). Rats were ganglion-blocked with mecamylamine 0.25 mg/kg i.v. Mean resting values (± SEM) for BP before and after ganglion blockade were 132 ± 5 and 108 ± 5 mmHg in the SHR, and 99 ± 2 and 85 ± 2 mmHg in the WKY rats respectively. Following ganglion blockade, mean regional vascular conductance values (VC, ± SEM, units 100[kHz/mmHg]) in SHR and WKY rats respectively were : REN VC, 2.38 ± 0.39 and 3.01 ± 0.48 units; MES VC, 2.46 ± 0.30 and 3.99 ± 0.44* units; CAR VC, 0.44 ± 0.07 and 0.60 ± 0.11 units; and HQ VC, 1.31 ± 0.13 and 2.81 ± 0.21* units.

Endothelin (porcine, Peptide Institute, Japan; 0.1 - 1 nmol/kg i.v.) elicited qualitatively similar biphasic responses in both SHR and WKY rats. The initial effect was a dose-related fall in BP; the mean changes (mmHg) with each dose in SHR and WKY rats respectively were : 0.1 nmol/kg, -20 ± 3 and -19 ± 2; 0.3 nmol/kg, -24 ± 2 and -18 ± 2*; 1 nmol/kg, -33 ± 5 and -24 ± 2*. Concomitant with these falls in BP were <u>increases</u> in CAR and HQ VCs but <u>decreases</u> in REN and MES VCs. With the highest dose, 1 nmol/kg, in both groups of rats the initial depressor effects were followed by marked increases in BP associated with substantial falls in VC in all four beds. There were large differences between SHR and WKY rats with respect to the magnitudes of the initial vasodilator responses of the CAR and HQ beds. The % increases in CAR VC in SHR and WKY rats respectively were : 0.1 nmol/kg, 166 ± 21 and 78 ± 17* ; 0.3 nmol/kg, 227 ± 25 and 66 ± 19* ; 1 nmol/kg, 389 ± 36 and 168 ± 26*. In the HQ bed, % increases in VC were : 0.1 nmol/kg, 103 ± 11 and 99 ± 11; 0.3 nmol/kg, 141 ± 11 and 107 ± 14* ; 1 nmol/kg, 249 ± 30 and 175 ± 12* in SHR and WKY rats respectively. Little difference was noted between the concomitant REN and MES vasoconstrictor responses in the 2 groups. In similar experiments, bolus injections of acetylcholine (0.01 - 1 µg/kg) or nitroprusside (0.3 - 10 µg/kg) revealed no marked differences between rat strains in the HQ or CAR responses.

Thus, our results show clear differences between SHR and WKY rats with respect to the regional haemodynamic effects of endothelin. These cannot be explained on the basis of different baseline parameters and hence provide support for the idea that altered sensitivity to endothelin may be a feature of certain cardiovascular diseases (Tomobe et al, 1988).

* different to SHR value, $p < 0.05$, unpaired 't' test.

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PHARMACOLOGICAL AND BIOCHEMICAL COMPARISON OF TRH AND RX77368 IN PITUITARY GH_3 CELLS

A.M.McDermott*, G.P.Wilkin, P.M.Taylor and S.L.Dickinson, Department of Biochemistry, Imperial College, London, SW7 2AZ. Department of Chemistry, Reckitt & Colman, Hull, HU8 7DS and Reckitt & Colman Psychopharmacology Unit, The Medical School, Bristol, BS8 1TD.

The tripeptide thyrotropin releasing hormone (TRH), which exists throughout the central nervous system, is known to prevent neuronal damage (Faden et al. 1981), promote recovery following spinal trauma (Freedman et al. 1986) and improve the weakness and spasticity which occurs in motorneurone disease (MND, Engel et al. 1983) although its usefulness is limited by its short biological half life. Recently, a biologically stable analogue of TRH (RX77368, pGlu-His-3,3'-dimethyl-ProNH_2) with improved *in-vivo* potency (Metcalf 1982) has similarly been shown to be of value in MND (Guiloff et al. 1987) yet has lower affinity at spinal cord receptors (Hawkins et al. 1986). The characteristics of receptor binding and the ability to stimulate phosphoinositide (PI) hydrolysis of RX77368 and TRH have therefore been directly compared *in-vitro* using cultured pituitary GH_3 cells which possess TRH receptors (Hinkle & Tashjian 1973).

GH_3 cells were grown to confluency on poly-l-lysine coated 13 mm glass coverslips in 24 well plates. For receptor binding studies, cells were washed and incubated at 37 deg.C with medium (Ham's F10 supplemented with 25mM Hepes, 5mM $MgSO_4$, 14mM $NaHCO_3$, 400 µg/ml BSA) containing either [^{3}H]-TRH (119.2 Ci/mmol) or [^{3}H]-RX77368 (32.7 Ci/mmol). Specific binding was defined in the presence of 10 or 100 µM unlabelled TRH. Incubation was terminated by rapid aspiration and washing with 3x1.0 ml volumes of ice cold medium. For PI assays, cells were prelabelled for 48hr. with [^{3}H]-myo-inositol (40-80 Ci/mmol). Cells were then washed and incubated at 37 deg.C for 30 min. with medium (as above) containing 10mM LiCl followed by a further 30 min. containing peptides. Total inositol phosphates were measured as described by Cholewinski et al. (1988).

Preliminary studies showed that both [^{3}H]-TRH and [^{3}H]-RX77368 bound to GH_3 cells reaching equilibrium between 30 to 45 mins. Hanes analysis of [^{3}H]-TRH and [^{3}H]-RX77368 binding (37 deg.C, 60 min.) indicated that it was saturable with a single component. Kd and Bmax values for TRH and RX77368 were 16 nM, 227 fmol/mg protein and 120nM, 151 fmol/mg protein respectively (Non specific binding was less than 35%). The order of potency of a series of TRH-analogues to inhibit binding were as follows: vs [^{3}H]-TRH; RX74355 > TRH >= MK771 >= CG3703 > RX77368 > CG3509: vs [^{3}H]-RX77368; RX74355 >= TRH > MK771 >= CG3703 > RX77368 > CG3509. Both TRH and RX77368 stimulated PI hydrolysis in a dose dependent fashion. EC_{50} values for TRH and RX77368 were 1.7 ± 0.6 nM (mean ± sem) and 64 ± 10 nM respectively.

These data, in GH_3 cells, indicate that TRH binds with high affinity to a single site and potently stimulates PI hydrolysis. RX77368 binds to a pharmacologically similar site but with 8 times lower affinity and with 38 times lower potency at stimulating PI hydrolysis. These *in-vitro* data thus suggest that the increased *in-vivo* potency of RX77368 may be due largely to its enhanced metabolic stability.

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EFFECTS OF TMB-8 (AN INHIBITOR OF INTRACELLULAR CALCIUM LIBERATION) ON NEUROHORMONE RELEASE FROM THE RAT NEUROHYPOPHYSIS

Racké K., Becker D., Niebauer J., Hering B., Berrino L.*, Marmo E.* & Haas U. Department of Pharmacology, University of Mainz, F.R.G.; *Institute of Pharmacology & Toxicology, 1st Faculty of Medicine, University of Naples, Italy.

Depolarization-induced calcium influx is a key event in the stimulus-secretion coupling of the release of neurotransmitters and neurohormones. Nevertheless, the process of stimulus-secretion coupling is still poorly understood. Since the work of Douglas and Poisner (1964), which demonstrated the calcium-dependence of neurosecretion, the isolated neurohypophysis has often been used as a tool for studying the process of stimulus-secretion coupling. The release of vasopressin and oxytocin shows a marked frequency-dependent facilitation, i.e. low frequencies (< 5 Hz) are markedly less effective than higher frequencies in inducing hormone secretion (e.g. Dutton & Dyball, 1979). There is evidence for a frequency-dependent facilitation of the terminal impulse invasion (Hobbach et al. 1986; Dyball et al., 1988), but additional mechanisms may be involved in the facilitation of neurohormone release. In the present experiments it was tested whether liberation of calcium from intracellular stores may contribute to the calcium signal which triggers the exocytotic hormone release. Therefore, the effects of TMB-8 (3,4,5-trimethoxybenzoic acid 8-(diethylamino)octyl ester) which inhibits the release of calcium from intracellular stores, on the neurohypophysial release of oxytocin and vasopressin was studied.

Isolated neural lobes with their stalks fixed to a platinum wire were superfused with Krebs solution. Oxytocin and vasopressin released into the medium were determined by RIAs. After 57 min of superfusion hormone release was evoked by electrical stimulation of the pituitary stalk (450 pulses 0.2 ms, 30 mA at a frequency of 3 or 15 Hz) or by raising the potassium concentration in the superfusion medium to 45 mM for 30 min. The test drug TMB-8 (10-100 µM) was added to the superfusion medium from the onset of superfusion.

In control experiments, stimulation at 15 Hz evoked the release of 528 ± 85 µU vasopressin and 757 ± 109 µU oxytocin (means ± s.e.m, n=10), stimulation at 3 Hz caused only a marginal hormone release (45 ± 8 µU vasopressin and 61 ± 11 µU oxytocin, n=11). The evoked release of vasopressin and oxytocin was prevented after removal of calcium from the medium. Neurohormone release evoked by stimulation at 15 Hz was reduced by TMB-8 in a concentration-dependent manner. In the presence of 50 µM TMB-8, the evoked release of oxytocin was reduced by 90 ± 3 % and that of vasopressin by 94 ± 2 % (n=6).

On the other hand, neurohormone release evoked by 'high potassium' was only slightly affected by TMB-8. In control experiments, superfusion with 45 mM potassium induced the release of 2855 ± 548 µU vasopressin and 6844 ± 1200 µU oxytocin (n=5). In the presence of 50 µM TMB-8 the 'high potassium'-induced release of vasopressin and oxytocin was reduced by only about 20-30 %.

In conclusion, the present experiments suggest that liberation of intracellular calcium, possibly triggered by the influx of extracellular calcium, may be involved in the impulse-induced, frequency-dependent neurohormone secretion.

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DIFFERENTIAL RELEASE OF PEPTIDES FROM CULTURED ISOLATED FETAL RAT HYPOTHALAMIC NEURONS IN RESPONSE TO NEUROTRANSMITTERS

G Gillies (introduced by J C Buckingham), Department of Pharmacology, Charing Cross & Westminster Medical School, Fulham Palace Road, London W6 8RF.

The control of hypothalamic neuron function has been studied, largely indirectly *in vivo* or directly in short term studies on hypothalamic fragments *in vitro*. In order to study longer term influences of environmental factors on the response of hypothalamic peptidergic neurons, a model of cultured hypothalamic neurons has been developed. Briefly, the cells from 18 day fetal hypothalami are dispersed mechanically and enzymatically. The cells are then grown in 35mm tissue culture dishes which were pre-treated with poly-L-lysine, and maintained in a defined medium for up to six weeks. The basal and stimulated release of somatostatin (SRIF), vasopressin (VP) and corticotrophin releasing factor (CRF-41) were followed using immunoassays.The responses to non- depolarising stimuli such as elevated concentrations of K^+ in isotonic medium, or veratridine, have already been studied and shown to be dose and Ca^{++}-dependent (Clarke et. al., 1987; Clarke and Gillies 1988).

We have now studied the peptide responses to specific neurotransmitters. Acetylcholine (Ach), which has been reported to stimulate release of CRF bioactivity *in vivo* and *in vitro*, had negligible effects on the release of all three peptides on day 15 of culture. However, by day 29 a significant stimulation above basal was observed at 10^{-10}M with maximal effect at 10^{-9} M for both CRF-41 and AVP. These effects were mimicked by carbachol. No effect was observed on SRIF release. Serotonin (5HT), another substance with reputedly stimulatory effects on bioactive CRF release was active in stimulating only immunoreactive CRF-41release during the early stages of the cultures at a concentration of 10^{-6}M. However, by day 30, an enhanced response was observed over the concentration range 10^{-10}-10^{-7}M. No consistent effects of 5HT were observed on AVP or SRIF. The inhibitory neurotransmitter gamma aminobutyric acid (GABA) was found to be effective only at high concentrations (10^{-5}-10^{-4}M) in inhibiting the release of all three peptides. Both bicuculline and picrotoxin were potent stimulators of the release of all three peptides, suggesting a high GABAergic tone in this preparation. Addition of 10^{-4} M GABA prior to simulation with bicuculline (10^{-7}-10^{-4} M) failed to significantly reduce the effects of bicuculline, which could suggest a possible non-specific effect of bicuculline. However, a rapid uptake of GABA by glial cells present in the cultures cannot be discounted and could account for the high concentrations of GABA required to show inhibition.

In conclusion, cultured 18 day fetal rat hypothalamic peptidergic neurons exhibit a maturing responsiveness and sensitivity to Ach and 5HT with time in culture. As the hypothalamo-pituitary-adrenal axis is hyporesponsive to stress for a short period after birth, it is proposed that this effect is due partly to the immaturity of the hypothalamic neurons secreting CRF (viz: CRF-41 and vasopressin). Furthermore, the increase in responsiveness *in vitro* with time may reflect a pattern of maturation which was pre-set *in vivo* which may continue under the right conditions *in vitro*. The three types of peptidergic neurons studied respond differentially to Ach and 5HT, which argues against a non-specific effect, and responses in this system mimic those which have been observed *in vivo* and in intact preparations of hypothalamic fragments *in vitro* (Buckingham and Hodges, 1979; Hillhouse et al., 1975; Ganong W., 1980). It is proposed, therefore, that this is a valid model for the direct study of the pharmacology of maturing neuron.s *in vitro*.

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IN VIVO RELEASE OF OPIOID PEPTIDES IN THE RAT CNS: MICRODIALYSIS COMBINED WITH A PLATE RIA

N.T. Maidment*, D.R. Brumbaugh, E. Erdelyi, V.D. Rudolph, J.D. Barchas & C.J. Evans, Department of Psychiatry & Behavioural Sciences, Medical School, Stanford University Medical Centre, Stanford, CA 94305 U.S.A.

The application of intracerebral dialysis to neuropeptides has proven difficult due to their relatively low extracellular concentrations (Lindefors et al, 1987). The IN VIVO release of opioid peptides has thus far only been possible with the more disruptive push-pull perfusion technique (Cesselin et al, 1981) . We report the successful measurement of basal and evoked release of opioid peptides from the rat globus pallidus / ventral pallidum using microdialysis combined with a highly sensitive radioimmunoassay (RIA).

Dialysis probes were constructed with a concentric cannulla design using 4mm lengths of several types of dialysis membranes. Their ability to recover met- and leu-enkephalin in solution was examined and compared to that of commercially available probes (Carnegie Medecin). Of the custom made probes, cuprophan (6.5 μm wall thickness) and polyacrylonitrile membranes proved the most successful with relative recoveries of approximately 6 -10%. The commercial probes demonstrated similar recovery values and were used in these preliminary IN VIVO studies.

Male Sprague Dawley rats, anaesthetised with halothane/N_2O throughout the experiment, were implanted with a dialysis probe in the globus pallidus / ventral pallidum and perfused with artificial CSF at a flow rate of 2.7μl/min. After a post-implantation period of approximately 1 hour, samples were collected on ice at 20 or 30 min. intervals and stored at -80^{o} C prior to assay. The RIA procedure involved the use of an antibody directed against the acetylated form of the N-terminal YGGF sequence present in all known active endogenous opioid peptide fragments and thus is termed a "universal opioid assay". The assay is unique in that the competition takes place on solid phase with the antibody bound via protein A to Immulon II coated 96 well plates. Separation of bound from free trace is thus accomplished by simple washing of the plates after which the wells are physically separated and counted. This system results in negligible non - specific binding and when HPLC-purified ^{125}I labelled trace is used IC_{50} values of 1 fmol are obtained with a limit of detection of approx. 0.2 fmol.

Basal release produced approx. 1.5 fmol of immunoreactivity per 30 min. sample. Perfusion with 100 mM K^+ for 2 min. of a 30 min. sample period increased the release to 43.9 fmol +/-12.4 SEM (n=4). A second stimulation 3 hours later also resulted in elevated levels with an S2:S1 ratio of 0.64 +/-0.03. The second stimulation was completely blocked by perfusion of a 10 mM EGTA-containing CSF, basal release remaining unaffected. Repeated stimulation with 50 mM K^+ at 1 hour intervals over a 6 hour period produced progressively smaller elevations in immunoreactivity demonstrating the predicted depletable nature of the release. Incorporation of veratridine (50 μM) in the perfusion medium for 2 min. of a 30 min. collection period increased the release to 16.8 fmol +/-0.5 (n=4) with an S2:S1 ratio of 0.62 +/-0.17 after a 3 hour interval. The second stimulation was prevented by perfusion with 2 μM tetrodotoxin-containing artificial CSF.

The majority of the immunoreactive material obtained under both basal and stimulated conditions co-chromatographed with met- and leu-enk with smaller and less consistent peaks co-eluting with the tetrapeptide YGGF, the heptapeptide met-enk RF and the octapeptide met-enk RGL on reverse phase HPLC. We are currently extending our studies to the freely moving rat and anticipate that this system will prove invaluable for investigating the regulation of opioid peptide release IN VIVO.

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INVOLVEMENT OF ADENOSINERGIC MECHANISMS IN THE POTENTIATION OF HEAD TWITCHES BY DIAZEPAM

P.C. Moser, Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg Cédex, France.

It has previously been shown that moderate to high doses of benzodiazepines either have no effect on , or potentiate, head-twitches in mice depending on wether the head-twitches were induced by 5-hydroxy-L-tryptophan (although see Handley & Singh, 1985) or by 5-methoxy-N,N-dimethyltryptamine (5-MeODMT) respectively (Moser & Redfern, 1988). The potentiation of 5-MeODMT-induced head-twitches could not be antagonised by flumazenil and is therefore unlikely to involve benzodiazepine receptors. As benzodiazepines are also able to inhibit adenosine uptake (at concentrations higher than those active at benzodiazepine receptors; Phillis *et al.*, 1980), the involvement of adenosinergic mechanisms in their potentiation of 5-MeODMT-induced head-twitches has been examined.

Head-twitches were induced in male Swiss albino mice (Charles River, France) using 5-MeODMT, as previously described in detail (Moser & Redfern, 1988). Mice were observed between 1 and 4 min post-5-MeODMT and the number of head-twitches observed in this period were recorded. The results are presented as mean ± s.e.mean for groups of 6 - 8 mice and Dunnett's t-test was used to compare data from treated groups with the approprite control group. All compounds were administered i.p. and unless otherwise stated a 30 min pretreatment time was used.

In confirmation of previous results, diazepam (0.5 - 16 mg kg^{-1}) dose-dependently potentiated head-twitches induced by 5-MeODMT (2.5 mg kg^{-1}) from 4.7 ± 0.4 in saline pretreated mice to a maximum of 14.0 ± 1.4 following 4 mg kg^{-1} diazepam, and the potentiation obtained with diazepam (4 mg kg^{-1}) was not inhibited by the benzodiazepine antagonist flumazenil (10 - 100 mg kg^{-1}). Indeed, flumazenil was itself found to potentiate 5-MeODMT-induced head-twitches although this only reached significance at 30 mg kg^{-1} (saline: 4.5 ± 0.9; flumazenil 9.0 ± 1.4; $P < 0.05$). In contrast, the effect of diazepam (4 mg kg^{-1}) was dose-dependently inhibited by the adenosine receptor antagonist caffeine (0.3 - 30 mg kg^{-1}), with an ED_{50} of 5.8 mg kg^{-1}. Over the same dose-range caffeine had no significant effect against 10 mg kg^{-1} 5-MeODMT; a dose which induced a similar number of head-twitches (5-MeODMT 2.5 mg kg^{-1} with diazepam 4 mg kg^{-1}: 11.7 ± 1.3; 5-MeODMT 10 mg kg^{-1}: 14.0 ± 1.2).

Other compounds that have been reported to inhibit adenosine uptake were also tested for their ability to potentiate head-twitches induced by 5-MeODMT (2.5 mg kg^{-1}). Both dipyridamole (3 - 100 mg kg^{-1}) and flunarizine (0.3 - 10 mg kg^{-1}) failed to affect head-twitches but papaverine (4 - 64 mg kg^{-1}, 10 min pretreatment) had a clearly biphasic effect. From 4 - 16 mg kg^{-1} a dose-related potentiation was observed (saline: 5.0 ± 0.8; 16 mg kg^{-1}: 11.3 ± 1.6; $P < 0.01$), but this effect reversed at higher doses such that at 64 mg kg^{-1} a significant inhibition was obtained (1.6 ± 1.4; $P < 0.05$). Marked sedation was also observed at the higher doses. The adenosine agonists 5'-(N-ethyl)carboxamido-adenosine (0.25 - 30 μg kg^{-1}), N^6-cyclohexyl-adenosine (10 - 300 μg kg^{-1}, 15 min pretreatment) and (-)-N^6-(R-phenylisopropyl)-adenosine (1 - 100 μg kg^{-1}, 15 min pretreatment) all failed to potentiate head-twitches induced by 5-MeODMT (2.5 mg kg^{-1}). Head-twitches were inhibited and marked sedation was seen at the higher doses of all three of these compounds.

These preliminary results suggest that the potentiation of 5-MeODMT-induced head-twitches observed following benzodiazepine treatment is the result of inhibition of adenosine uptake, a property shared by benzodiazepine agonists and antagonists. They support the proposal that benzodiazepine receptors are not involved in this response and suggest that benzodiazepines, at doses that are commonly used, can have behavioural effects mediated via inhibition of adenosine uptake. However, the use of antagonists more specific than caffeine is obviously required, as well as an explanation for the lack of effect of dipyridamole and flunarizine, although neither of these compounds is very specific for the adenosine uptake site. The lack of effect of the direct adenosine agonists may indicate that the potentiation of head-twitches requires uptake inhibition at a site where adenosine levels are already high.

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REPEATED ADMINISTRATION OF TRICYCLIC ANTIDEPRESSANTS DOES NOT ALTER THE ANTIDEPRESSANT-LIKE EFFECT OF 8-OH-DPAT IN MICE

G.P. Luscombe*, L.J. Hutchins and W.R. Buckett, Research Department, The Boots Company PLC., Nottingham, NG2 3AA

Repeated administration of antidepressants to mice attenuates the hypothermia induced by the 5HT-1A receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OHDPAT), a response mediated by presynaptic 5HT-1A receptors (Goodwin et al, 1985). 8-OHDPAT also has the antidepressant-like effect of increasing the mobility of mice in the Porsolt test (Luscombe et al, 1988), a response involving 5HT-1A receptors which do not appear to be presynaptically located (Luscombe et al, 1989). Repeated treatment with 8-OHDPAT attenuates the hypothermia but not the antidepressant-like activity of 8-OHDPAT in mice (Luscombe et al, 1989). We now report that the antidepressant-like effect of 8-OHDPAT in mice is also unaffected by repeated administration of tricyclic antidepressants.

Male CD1 mice (Charles River; 22-24g at initiation of treatment) were orally administered either amitriptyline (3-30 mg/kg), desipramine (3-30 mg/kg), dothiepin (10-100 mg/kg) or deionised water once daily for 10 days. Twenty three hours after the final dose, either 8-OHDPAT (3 mg/kg) or saline was administered subcutaneously. One hour after treatment, the mobility of mice in the test situation was measured as previously described (Luscombe et al, 1988).

The antidepressant-like increase in mobility induced by 8-OHDPAT (3 mg/kg s.c.) was not altered by 10 day pretreatment with any of the antidepressants (Table 1). Mobility following saline administration was similar in mice pretreated for 10 days with either antidepressants or deionised water.

TABLE 1 Effect of repeated administration of tricyclic antidepressants on the 8-OHDPAT-induced increase in the mobility of mice in the Porsolt test

Treatment	10 day antidepressant dose (mg/kg p.o.) + acute treatment (s.c.) 0 + SAL	0 + 8OH	3 + 8OH	10 + 8OH	30 + 8OH	100 + 8OH
	Mobility (mean ± s.e. mean; arbitrary units)					
Amitriptyline	217 ± 39	459 ± 52**	412 ± 55	486 ± 53	478 ± 55	NT
Desipramine	246 ± 27	377 ± 38*	418 ± 48	438 ± 41	464 ± 32	NT
Dothiepin	222 ± 37	392 ± 39**	NT	450 ± 45	410 ± 40	386 ± 67

** P<0.01, * P<0.05 vs 0 + SAL treatment group (William's test). NT: Not tested. n = 10-15 mice/treatment. SAL = Saline. 8OH = 8-OHDPAT (3 mg/kg s.c.).

The lack of effect of repeated tricyclic antidepressant administration on the antidepressant-like activity of 8-OHDPAT in mice compared to the reported ability of such treatments to attenuate 8-OHDPAT-induced hypothermia in mice (Goodwin et al, 1985), suggests that the two responses may be mediated by distinct groups of receptors. Since 8-OHDPAT-induced hypothermia in mice involves presynaptic 5HT-1A receptors (Goodwin et al, 1985), these sites would not appear to be involved in the antidepressant-like effect of 8-OHDPAT in mice. In addition, the 5HT-1A receptors mediating the 8-OHDPAT antidepressant-like response seem unaffected by repeated antidepressant treatment.

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DRUG PROFILE OF L-DOPA IN PLASMA AND MUSCLE FOLLOWING CHRONIC TREATMENT OF RATS WITH L-DOPA PLUS CARBIDOPA

S. Rose*, P. Jenner & C.D. Marsden, MRC Movement Disorders Research Group, University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

Chronic treatment of Parkinson's disease with L-DOPA plus a peripheral decarboxylase inhibitor leads to an unpredictability of drug response. One reason might relate to an alteration in L-DOPA levels in plasma or to its storage in muscle following its chronic administration. We present an initial report on the pharmacokinetic profile of L-DOPA in rats treated chronically with L-DOPA plus carbidopa compared with age-matched control groups.

Male Wistar rats (125 ± 2 g) received either L-DOPA plus carbidopa (LD/CD; 176-210 mg/kg/day plus 22-26 mg/kg/day) or carbidopa (CD; 19-26 mg/kg/day) alone for 52 weeks via their daily drinking water. Control rats received normal drinking water. Animals were withdrawn from chronic treatment for 4 days and fasted for 16 hours prior to experimentation. Some rats from each group were killed for measurement of hepatic DOPA decarboxylase activity using a radioenzymatic assay. Other rats, previously cannulated in the descending aorta, were treated with L-DOPA (50 mg/kg i.a. or p.o.). Blood samples were withdrawn at intervals upto 8 h via the aortic cannula. A further group of animals were killed at intervals upto 8 h following administration of L-DOPA (200 mg/kg p.o.). Biceps muscle and blood from the neck stump were collected. Plasma and tissue L-DOPA levels were measured by HPLC with electrochemical detection.

The Km and Vmax values for hepatic DOPA decarboxylase activity were unaltered after CD treatment, however, LD/CD treatment tended to increase the Km and Vmax of this enzyme. LD/CD or CD treatment had no effect on the pharmacokinetics of intra-aortically administered L-DOPA (Table 1). In contrast, CD treatment produced a decreased $t_{\frac{1}{2}}$, Vd and Cl but no change in AUC after oral administration of L-DOPA compared to control values. LD/CD treatment produced an increased $t_{\frac{1}{2}}$ compared to CD alone, and an increased Vd and Cl, and no change in AUC compared to CD and control. Plasma L-DOPA levels were similar in all groups between 0-8 h after administration of L-DOPA (200 mg/kg). Over the 8 h period there was no difference in the accumulation of L-DOPA into muscle between the three groups ($p > 0.05$; 2 way ANOVA).

Table 1 The effect of LD/CD and CD treatment on L-DOPA pharmacokinetics

		Control	CD	LD/CD
Oral administration	$t_{\frac{1}{2}}$ (h)	0.75 ± 0.03	0.62 ± 0.04*	0.90 ± 0.06^{+}
	Vd (L/kg)	4.29 ± 0.42	2.01 ± 0.43*	8.62 ± 0.81*$^{+}$
	Cl (L/kg/h)	3.95 ± 0.36	2.25 ± 0.47*	6.10 ± 0.28*$^{+}$
	AUC (mg.h/L)	22.6 ± 3.3	26.0 ± 2.6	19.33 ± 2.97
i.a. administration	$t_{\frac{1}{2}}$ (h)	0.56 ± 0.08	0.59 ± 0.05	0.63 ± 0.04
	Vd (L/kg)	3.35 ± 0.49	2.89 ± 0.23	4.97 ± 0.81
	Cl (L/kg/h)	4.40 ± 0.62	3.57 ± 0.56	5.64 ± 1.10
	AUC (mg.h/L)	100.8 ± 18.0	131.7 ± 41.6	75.11 ± 14.4

* $p < 0.05$ compared with control; $^{+}$ $p < 0.05$ compared with CD (Dunn's test).

These data suggest that chronic CD and LD/CD to rats altered the pharmacokinetics of orally but not intra-aortically administered L-DOPA in rats. This could reflect changes in enzyme activity, changes in the regulation of compartmental uptake systems and/or changes in regional blood flow which may induce changes in plasma clearance values.

THE EFFECT OF FOETAL pH AND MATERNAL HYPOTENSION ON TRANSFER OF ANTIPYRINE, BUPIVACAINE AND PETHIDINE ACROSS THE RABBIT PLACENTA

R. J. Carson, D. G. Gaylard & F. Reynolds. Anaesthetic Unit, UMDS St Thomas' Hospital, London SE1 7EH.

In the rabbit placenta perfused in situ, clearance (mass of drug crossing placenta/maternal concentration) of lipid soluble drugs is dependent on umbilical blood flow and differential protein binding (Hamshaw-Thomas & Reynolds 1985). However, weak electrolytes are also affected by pH gradients and fetal acidosis is associated with an increase in fetal:maternal (F/M) bupivacaine ratio in intact sheep (Kennedy et al 1979) and man (Datta et al 1981), while the influence of maternal blood flow is harder to establish (Vella and Reynolds 1987). We have therefore studied the effect of umbilical perfusate pH and maternal hypotension on the F/M ratios of antipyrine (an index of placental exchange), bupivacaine and pethidine, both basic drugs that are commonly used in labour.

Antipyrine (12 mg/h) bupivacaine and pethidine (3.75 mg/h) were given intravenously, after loading doses, to seven anaesthetised NZW does at 26-29 days gestation. A single placenta in each doe was perfused at 1 ml/min with Krebs bicarbonate buffer containing dextran, at normal and reduced pH (7.02). Following return to normal pH, maternal blood was withdrawn (mean total 48 ml) so as to reduce her mean arterial pressure by 35%, and subsequently re-transfused. Drug concentrations were measured by gas chromatography in duplicate samples of maternal arterial plasma and effluent perfusate during control perfusion (1) acid perfusion (2) a second control period (3) hypotension (4) and after re-transfusion (5).

Table: F/M ratios. (mean $\pm$ standard deviation) during each of the above phases.

	1	2	3	4	5
Antipyrine	0.795$\pm$0.145	0.520$\pm$0.262	0.561$\pm$0.257 *	0.127$\pm$0.065 *	0.270$\pm$0.102
Bupivacaine	0.054$\pm$0.013 *	0.133$\pm$0.032 *	0.060$\pm$0.015	0.096$\pm$0.053	0.085$\pm$0.044
Pethidine	0.726$\pm$0.247 *	1.31$\pm$0.421 *	0.501$\pm$0.232 *	0.260$\pm$0.125	0.324$\pm$0.068

* $P < 0.03$, by paired t test.

The F/M ratio of antipyrine, which is poorly ionised, was unaffected by perfusate pH, but fell significantly during maternal haemorrhage, suggesting that maternal blood flow was reduced by this manoeuvre. The F/M ratios of both bupivacaine and pethidine rose with acidotic umbilical perfusate, demonstrating that ion trapping can actually increase placental clearance as well as equilibrium F/M ratios for both these drugs. While clearance of pethidine fell during maternal haemorrhage, that of bupivacaine was not significantly affected, suggesting more complete extraction from maternal blood of this more highly bound and lipid soluble drug.

Thus in the acidotic fetus the transfer of both pethidine and bupivacaine can be expected to be enhanced, while in the presence of maternal hypotension reduced transfer of bupivacaine cannot be expected.

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SKF 38393 AND SCH 23390 ANTAGONISE THE EFFECTS OF LY 171555 BUT NOT OF APOMORPHINE IN MARMOSETS

P-A Loeschmann*, P Jenner and C D Marsden, MRC Movement Disorders Research Group, University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

In MPTP-treated common marmosets the D-2 agonists LY 141865 or LY 171555, but not the D-1 agonist SKF 38393, reverses motor deficits and enhances locomotor activity (Nomoto et al, 1985; 1988). Indeed, SKF 38393 dose dependently inhibits the motor effects of LY 171555 in MPTP-treated marmosets (Nomoto et al, 1988). This data argues against an interaction of D-1 and D-2 systems in primates similar to that reported in rodents (Waddington & O'Boyle, 1987). We now report on the effect of D-1 and D-2 agonist and antagonist drugs in normal common marmosets.

Groups of four normal common marmosets were treated with a range of doses of the mixed D-1/D-2 agonist apomorphine (0.18, 0.75 and 1.5 mg/kg s.c.), the D-2 agonist LY 171555 (0.15, 0.3 and 0.6 mg/kg i.p.), the D-1 agonist SKF 38393 (1.25, 2.5 and 5 mg/kg i.p.), the D-1 antagonist SCH 23390 (1.25 and 5 mg/kg i.p.) alone and in combination. When treated with dopamine agonists, the animals received the peripheral D-2 antagonist domperidone (2 mg/kg p.o.) to prevent nausea or vomiting. Locomotor activity was monitored for 2 h in home cages equipped with infrared photo cells.

Table 1 Motor activity in common marmosets

	Mean counts (± 1 SEM) in 120 min (n = 4)			
	Vehicle	1.25	2.5	5 mg/kg i.p.
SKF 38393	850 (391)	891 (364)	352 (115)	253 (128)
SCH 23390	1210 (1017)	455 (373)	ND	57 (25)
APO 1.5 mg/kg s.c.				
+ SKF 38393	4299 (496)	3777 (263)	3152 (399)	3306 (480)
+ SCH 23390	2672 (627)	2828 (817)	3700 (1176)	2460 (499)
LY 171555 0.6 mg/kg i.p.				
+ SKF 38393	4548 (740)	3483 (622)	2355 (191)	1877 (691)
+ SCH 23390	2790 (724)	3029 (910)	2537 (1410)	1374 (643)

Both apomorphine and LY 171555 stimulated locomotor activity in a dose dependent manner. Motor activity was dose dependently inhibited by SKF 38393, SCH 23390 and raclopride. SKF 38393 or SCH 23390 had no effect on the locomotor activity induced by administration of apomorphine (1.5 mg/kg s.c.) (Table 1). Locomotor activitv induced by LY 171555 (0.6 mg/kg p.o.) was dose dependently inhibited by SKF 38393 (Table 1). In contrast only the highest dose of SCH 23390 (5 mg/kg i.p.) tested was effective. Raclopride (1.25 mg/kg i.p.) antagonised the effects of both apomorphine and LY 171555.

These results suggest differences in the organisation of D-1 and D-2 receptors mediated effects in the rodent compared to this primate species.

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DELAYED RECOVERY OF COMMON MARMOSETS AFTER COMBINED TREATMENT WITH MPTP AND DIETHYLDITHIOCARBAMATE

P-A Loeschmann*,P Jenner and C D Marsden, MRC Movement Disorders Research Group, University Department of Neurology & Parkinson's Disease Society Research Centre, Institute of Psychiatry & King's College Hospital Medical School, Denmark Hill, London SE5, U.K.

Common marmosets develop motor deficits following 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment but gradually recover over a period of months and are resistant to further treatment with MPTP (Ueki et al, 1987). Diethyldithiocarbamate (DDC) increases the neurotoxicity of MPTP in mice (Corsini et al, 1985). So, we now investigate whether the administration of MPTP combined with DDC will induce persistent motor deficits in animals which have recovered from previous MPTP treatment.

Eight common marmosets were employed which one year previously had been treated with 10-12 mg/kg s.c. MPTP. These animals showed a reduced basal locomotor activity and a hyperactive response to dopamine agonists compared to untreated animals. But the mean disability score was reduced to 2 on a rating scale (0 = normal, 18 = maximal disability). Four animals were treated with 2 mg/kg MPTP s.c. on four consecutive days and four animals received the same dose of MPTP combined with 20 mg/kg DDC i.p., again for four days. The cumulative dose of 8 mg/kg MPTP s.c. is not sufficient to induce stable motor deficits in common marmosets.

Table 1 Effect of MPTP plus DDC on motor disability

	Mean disability score (n = 4)					
	Pre	Day 4	Day 7	Day 14	Day 21	Day 49
MPTP	3.0	16.5	15.3	5.8	3.8	3.0
MPTP + DDC	2.0	17.0	12.8	11.0	9.8	8.5

Animals treated with MPTP alone showed a marked increase in motor deficits at 4 and 7 days following the start of MPTP treatment. However, motor function then gradually recovered such that by day 49 the animals were indistinguishable from their pretreatment state. The marmosets treated with MPTP in combination with DDC also showed motor deficits at days 4 and 7 which were indistinguishable from those observed in animals receiving MPTP alone. In contrast, animals with MPTP plus DDC showed little recovery of motor function between days 7 and 49. At the end of the experiment these animals remained substantially affected by MPTP treatment.

We conclude that DDC enhances the neurotoxicity of MPTP in common marmosets, leading to a delayed recovery from the initial symptoms. Since DDC has many pharmacological effects it is difficult to decide which of those may be responsible for the enhancement of MPTP toxicity. The compound is a chelating agent and does inhibit dopamine-β-hydroxylase, superoxide dismutase and plasma amino-oxidases. Inhibition of peripheral MPTP metabolism by MAO may account for the effects seen.

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THE EFFECTS OF CHRONIC LITHIUM ON 5-HT RELEASE FROM RAT HIPPOCAMPAL SLICES

S.M.P. Anderson, M.S. Christodoulou and D.G. Grahame-Smith, MRC Unit of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford. OX2 6HE.

Lithium is used therapeutically in the treatment of manic-depression but the mechanisms responsible for its clinical effects are unclear at present. Short term lithium treatment reduces electrically evoked release of [^{3}H]-5-HT from rat striatal slices (Katz et al., 1968) and K^+-evoked release from hippocampal slices (Anderson et al., 1988). There is further evidence that lithium affects central 5-HT function by altering release of 5-HT from presynaptic terminals (Grahame-Smith and Green, 1974; Treiser et al., 1981; Friedman and Wang, 1988). We have studied the effects of chronic lithium administration on 5-HT release from rat hippocampal slices. We have also examined whether chronic lithium administration produces tolerance to the acute inhibitory effects of lithium on release of 5-HT from hippocampal slices.

Male Sprague-Dawley rats (150-250g) were fed a diet containing 0.1% lithium carbonate and control animals received ordinary chow. Food and water were freely available. After 21-28 d hippocampal slices were prepared from control and lithium-treated rats. Slices (250-250 μm) were loaded with [^{3}H] 5-HT (0.1 μM; specific activity 28.3 mCi/mmol) in oxygenated Hepes-Ringer buffer, pH 7.4, containing 50 μM pargyline. Slices were superfused at 0.5 ml/min in the buffer containing 0.5 μM citalopram. A 4-min pulse of buffer containing 35 mM K^+ ions was given after 28 min; when used lithium (1 - 10 mM) was added at the start of the perfusion. Fractions were collected every 4 min and [^{3}H]-5-HT measured by liquid scintillation counting. Plasma Li levels were measured by atomic absorption spectrophotometry.

Hippocampal slices from rats treated chronically with lithium showed no change in either loading pattern with (^{3}H)-5-HT or basal 5-HT release. However K^+-evoked release was increased by 25 ± 6%. When lithium was included in the superfusion medium of control hippocampi, 5-HT release was reduced in a dose-dependent manner by 45 ± 6% (1 mM) and 61 ± 5% (10 mM). In rats treated for 28 d with a lithium diet, there was no change in 5-HT release from hippocampal slices with either 1 or 10 mM Li^+ in the superfusion medium. Rats which had received a chronic lithium diet for 14 d were then withdrawn from it and received ordinary chow. After 14 d withdrawal K^+-induced 5-HT release from hippocampal slices was still resistant to inhibition by 1 or 10 mM Li^+. However after 28 d withdrawal, addition of lithium ions to the superfusion medium caused a dose-dependent reduction in K^+-stimulated release from hippocampal tissue by 27 ± 4% (1 mM) and 46 ± 5% (10 mM) showing that sensitivity to the inhibitory effects of lithium had returned.

K^+-induced release of 5-HT from rat hippocampal slices is increased by chronic lithium treatment; it also becomes remarkably insensitive to the inhibitory effects of exogenously applied lithium, and this insensitivity lasts at least 14 d after lithium withdrawal.

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EFFECTS OF LESIONING CENTRAL 5-HT AND NORADRENALINE CONTAINING NEURONES ON 8-OH-DPAT HYPOTHERMIA, MYDRIASIS AND HYPOACTIVITY

D. J. Heal, M. R. Prow, K. F. Martin and W. R. Buckett, Research Department, The Boots Company PLC, Nottingham NG2 3AA

8-Hydroxy-2-(di-n-propylamino)tetralin (8OH-DPAT) produces hypothermia in mice (Goodwin et al, 1985). Recently, Heal et al (1989) demonstrated that 8OH-DPAT also induced mydriasis and hypoactivity in mice. We found that mydriasis was 5-HT_{1A} mediated, but the receptors responsible for hypoactivity were less clearly identified. In addition, the results also suggested that 8OH-DPAT indirectly induced mydriasis by increasing the release of noradrenaline which activated postsynaptic α_2-adrenoceptors; this is analogous to the action of methamphetamine (Heal et al, 1988a). We have now lesioned central 5-HT and noradrenaline containing neurones to further elucidate the mechanisms underlying these three responses to 8OH-DPAT.

Adult male C57/Bl/601a mice (Olac, Bicester) weighing 20-30g were used. Pupil diameter was measured in conscious mice 10 min after injection of 8OH-DPAT (5 mg/kg) (Heal et al, 1988b). Hypoactivity induced by 8OH-DPAT (2 mg/kg) was scored 0-3 on 5 behavioural parameters at 10 min intervals for 60 min (Heal et al, 1981). Hypothermia was measured 20 min after 8OH-DPAT (0.5 mg/kg) using a rectal temperature probe. Drugs, excluding 5,7-dihydroxytryptamine (5,7-DHT), were injected i.p. dissolved in saline. 5,7-DHT (75 µg) or saline-ascorbate (4 µl) was injected intracerebroventricularly into halothane anaesthetised mice, pretreated with desipramine (5 mg/kg), using the method of Heal (1984). DSP-4 (100 mg/kg) was given to zimeldine (5 mg/kg) pretreated mice, on two separate occasions seven days apart. Results are given as mean ± s.e. Depletions of 5-HT, noradrenaline and dopamine, measured by HPLC with electrochemical detection, were, respectively:- DSP-4 lesioning, 10% (P<0.01), 74% (P<0.001), 15% (P<0.001) and 5,7-DHT lesioning, 70% (P<0.001), 34% (P<0.001), 1% (N.S.)

TABLE 1 Effects of 5,7-DHT or DSP-4 lesioning on 8OH-DPAT responses.

	Increase in pupil size (mm)	Hypoactivity (total score)	Temperature decrease(°C)
Sham lesion	0.13 ± 0.01	20.0 ± 1.0	1.3 ± 0.1
5,7-DHT	0.13 ± 0.01	7.1 ± 1.4**	0.5 ± 0.1***
Sham lesion	0.11 ± 0.01	18.6 ± 0.8	1.5 ± 0.2
DSP-4	0.03 ± 0.01***	9.9 ± 1.4**	1.2 ± 0.2

n = 7-9 observations. Significant reduction **P<0.01, ***P<0.001

The above data confirm that 8OH-DPAT mydriasis involves noradrenaline release, because it was abolished by DSP-4 lesioning. In addition, the 5-HT_{1A} receptors initiating this response are almost certainly postsynaptic, because 5,7-DHT lesioning was without effect. 8OH-DPAT hypoactivity remains enigmatic and the lesioning studies indicate that this effect is mediated via both 5-HT and noradrenaline containing neurones. The data also confirm the suggestion of Goodwin et al (1985) that the 5-HT_{1A} receptors responsible for hypothermia are probably autoreceptors on 5-HT neurones. In addition, the finding that DSP-4 lesioning had no effect further supports previous studies showing that noradrenergic neurones are not involved in the expression of this hypothermic response.

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IS DOWN-REGULATION OF 5-HT$_{1A}$ RECEPTOR FUNCTION A COMMON FEATURE OF THE ACTION OF ANTIDEPRESSANT DRUGS?

K.F.Martin*, I.Phillips, D.J.Heal & W.R.Buckett. Research Department, The Boots Co. PLC, Nottingham NG2 3AA.

The selective 5-HT$_{1A}$ receptor ligand, 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT) induces marked hypothermia in mice by an action on 5-HT$_{1A}$ autoreceptors (Goodwin et al 1985a). These authors have also reported that chronic administration of antidepressant drugs attenuated this response to DPAT and suggested that this may be a common effect of these treatments (Goodwin et al 1985b). However, the number of antidepressant treatments employed was limited and the effect of other types of psychotropic drug was not investigated. We have repeated these experiments and extended them to include other antidepressant and psychoactive drugs and now report that attenuation of DPAT-induced hypothermia is not a specific property of, nor exhibited by all, antidepressant drugs.

Groups of 10 adult male C57/Bl/601a mice (Olac, Bicester) weighing 20-30g were used. The following drugs, dissolved in saline, were given i.p. once daily for 14 days: sibutramine HCl(3 mg/kg), amitriptyline (10 mg/kg), zimeldine (10 mg/kg), dothiepin (30 mg/kg), mianserin (5 mg/kg), tranylcypromine (5 mg/kg), diazepam (2.5 mg/kg), haloperidol (0.5 mg/kg) or ketanserin (0.5 mg/kg). Controls were given saline (10 ml/kg). 5-HT$_{1A}$ receptor function was assessed 24 h after the first and the last treatment by measuring the hypothermia 20 min after DPAT (0.5 mg/kg s.c.) with a rectal probe. Each treatment group was compared with its own control group using Student's unpaired t-test. The control groups have been pooled in Table 1 for the purposes of clarity, since none were significantly different from the mean shown.

DPAT-induced hypothermia was attenuated after repeated administration of the antidepressants amitriptyline, dothiepin, mianserin and tranylcypromine but not zimeldine or sibutramine HCl(Table 1). Furthermore, this response was also reduced by chronic treatment with ketanserin and diazepam, but not haloperidol (Table 1). None of the drugs had any effect on DPAT-induced hypothermia after a single dose.

Table 1 The effect of psychoactive drugs on DPAT-induced hypothermia in mice

TREATMENT	DOSE (mg/kg i.p.)	DECREASE IN RECTAL TEMP.(°C) 1 DAY	14 DAYS
Control		1.29 ± 0.01(61)	1.34 ± 0.01(68)
Sibutramine HCl	3	1.12 ± 0.10(9)	1.25 ± 0.07(10)
Amitriptyline	10	1.11 ± 0.11(9)	0.87 ± 0.07(10)***
Zimeldine	10	1.33 ± 0.11(9)	1.26 ± 0.10(10)
Dothiepin	30	1.32 ± 0.12(9)	0.77 ± 0.09(10)***
Mianserin	5	1.18 ± 0.08(10)	0.83 ± 0.10(10)***
Tranylcypromine	5	1.40 ± 0.09(10)	0.64 ± 0.04(10)***
Diazepam	2.5	1.08 ± 0.05(10)	0.83 ± 0.07(10)***
Haloperidol	0.5	1.71 ± 0.12(9)	1.25 ± 0.09(10)
Ketanserin	0.5	1.28 ± 0.09(8)	1.06 ± 0.09(9)*

Values given are mean ± s.e.mean(n).
Significant difference from own control *P<0.05, **P<0.01, ***P<0.001.

These results are similar to the findings of Goodwin et al(1985b). However, the failure of zimeldine and sibutramine HCl to decrease DPAT-induced hypothermia demonstrates that down-regulation of 5-HT$_{1A}$ autoreceptor function is not a common feature of antidepressant drug action. Furthermore, the ability of a 5-HT$_2$ receptor antagonist, ketanserin and an anxiolytic, diazepam, to reduce DPAT-induced hypothermia indicates that this property is not restricted to antidepressant drugs.

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CARDIOVASCULAR EFFECTS OF 8-OH-DPAT IN THE ANAESTHETISED RABBIT

S.L. Shepheard*, D. Jordan[1] & A.G. Ramage, Academic Department of Pharmacology & [1]Department of Physiology, Royal Free Hospital School of Medicine, Hampstead, London NW3 2PF.

The selective 5-HT_{1A} receptor agonist 8-OH-DPAT has been shown to decrease blood pressure in rats, (Gradin et al, 1985; Fozard et al, 1987), in anaesthetised cats (Ramage & Fozard, 1987) and in conscious hypertensive dogs (Di Francesco et al, 1988). This effect has been demonstrated to be due mainly to a central sympathoinhibitory action of 8-OH-DPAT. The present experiments were carried out to investigate whether 8-OH-DPAT has a similar action in anaesthetised rabbits.

Rabbits were anaesthetised with urethane (1-1.5 g kg^{-1} i.v.), artificially ventilated and paralysed with vecuronium bromide (200 μg kg^{-1}). Simultaneous recordings were made of phrenic nerve activity (PNA), renal nerve activity (RNA), heart rate (HR) and arterial blood pressure (BP). A cumulative dose response curve was produced for 8-OH-DPAT (0.5-512 μg kg^{-1}, i.v, n=8). In a further 6 animals additional recordings were made of cardiac nerve activity (CNA). In these animals a single dose (32 μg kg^{-1}) of 8-OH-DPAT was given. This was also carried out in 5 pentobarbitone sodium (45 mg kg^{-1}) anaesthetised rabbits. Atropine sulphate (1 mg kg^{-1}) was given to determine the amount of vagal tone present both before and after the administration of 8-OH-DPAT.

In urethane anaesthetised rabbits cumulative doses of 8-OH-DPAT caused significant ($P<0.05$) dose related falls in mean blood pressure and heart rate reaching maxima of -17 ± 3 mmHg and -47 ± 7 beats min^{-1} (mean ± s.e. mean; n=8) respectively. The effects on nerve activity were variable; there was no significant dose related change in PNA, although this did tend to increase at the higher doses, and a significant decrease in RNA was only obtained at the lowest dose. In the single dose experiments 8-OH-DPAT caused a significant fall in mean blood pressure of 16 ± 4 mmHg and in heart rate of 28 ± 4 beats min^{-1} (n=6). There was no significant effect on PNA or RNA, however there was a significant decrease in CNA of a mean of -42 ± 6%. The only difference in the effects of a single dose of 8-OH-DPAT in the pentobarbitone anaesthetised rabbits was that the increase in PNA was significant. The 8-OH-DPAT-induced bradycardia was unaffected by atropine in all the experimental situations.

These results demonstrate that 8-OH-DPAT causes a bradycardia and hypotension in anaesthetised rabbits. However they differ from results obtained in the cat (Ramage & Wilkinson, 1988) in that the renal nerve in the cat was the most sensitive to the sympathoinhibitory action of 8-OH-DPAT. Furthermore these results show that in the rabbit the bradycardia produced by 8-OH-DPAT was not partly due to an increase in vagal tone, as has been previously demonstrated in the rat and cat. In this respect it is interesting that in the dog 8-OH-DPAT failed to cause bradycardia. Thus, it seems that there are a number of species differences in the role of central 5-HT_{1A} receptors in cardiovascular regulation.

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EVIDENCE OF THE INVOLVEMENT OF 5-HT_{1A} RECEPTORS IN THE CONTROL OF CARDIAC VAGAL MOTONEURONES IN THE ANAESTHETISED RAT

S.C.E. Sporton*, S.L. Shepheard, D. Jordan[1] & A. G. Ramage, Academic Department of Pharmacology & [1]Department of Physiology, Royal Free Hospital School of Medicine, Hampstead, London NW3 2PF.

Boutons containing 5-HT-like immunoreactivity have been shown to make synaptic contact with retrogradely labelled cardiac vagal motoneurones (CMV's) in the nucleus ambiguus of the cat, and when 5-HT or the 5-HT_{1A} agonist, 8-OH-DPAT, is microinjected into this nucleus a bradycardia is produced (Izzo et al., 1988). In the rat, 5-HT-like immunoreactive fibres (Steinbusch, 1981) have also been observed in the nucleus ambiguus and the dorsal motor vagal nucleus (DMVN) and both contain CMV's (Nosaka et al., 1979). Furthermore, in the conscious rat, i.c.v. injections of 5-HT evoked a bradycardia which was shown to be partly due to an increase in vagal drive to the heart and was suggested to be produced by activation of 5-HT_1-like receptors (Dalton, 1986). Thus, the aim of the present study was to investigate whether microinjection of 5-HT and 5-HT_{1A} agonists into the DMVN of the rat will evoke an increase in vagal tone.

Male Sprague-Dawley rats (250-375g) were anaesthetised with pentobarbitone sodium (90 mg kg^{-1}; i.p.) and were artificially ventilated following paralysis with decamethonium iodide (1 mg per animal). Recordings were made of arterial blood pressure, heart rate and phrenic nerve activity (PNA). All animals were given atenolol (1 mg kg^{-1}; i.v.). Microinjections (100 nl) of 5-HT (0.62 nmol), 8-OH-DPAT (1.2 nmol), flesinoxan (1.4 nmol), or vehicle were made into the right DMVN using glass five-barrelled microelectrodes. One barrel was filled with Wood's metal for electrical stimulations (40 Hz, 10-20 µA, 0.2 ms). The electrode tip was assumed to be in the DMVN if an instantaneous bradycardia was elicited by electrical stimulation and/or microinjection of glutamate. Injection sites were marked with pontamine sky blue dye for later histological verification.

Microinjections of 5-HT had variable effects on heart rate, producing a small bradycardia (n=2) or no change (n=1). 5-HT had no significant effects on blood pressure or PNA. Microinjection of 8-OH-DPAT or flesinoxan caused consistent significant ($p<0.05$) falls in heart rate of a mean of -11 ± 2 (n=5) and -10 ± 1 beats $min.^{-1}$ (n=6) respectively (mean $\pm$ s.e. mean) but had no significant effects on blood pressure. There were also significant increases in PNA of a mean of $38.9 \pm 9.9\%$ (n=5) and $31.9 \pm 6.5\%$ (n=6) respectively.

These results demonstrate that both 8-OH-DPAT and the structurally unrelated 5-HT_{1A} agonist flesinoxan caused a bradycardia when microinjected into the DMVN of the atenolol-pretreated, anaesthetised rat. Since the bradycardia could not be due to withdrawal of sympathetic tone it must presumably be the result of an increase in vagal drive to the heart. 8-OH-DPAT and flesinoxan both caused an increase in PNA. These results support the hypothesis that activation of 5-HT_{1A} receptors in the DMVN causes excitation of vagal motoneurones and also suggest that activation of these receptors results in an increase in central respiratory drive.

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HETEROGENEOUS 5-HT_{1D} BINDING SITES IN PORCINE BRAIN CAN BE DIFFERENTIATED BY GR43175

M.J. Sumner and P.P.A. Humphrey, Pharmacology Division, Glaxo Group Research Ltd., Ware, Herts. SG12 0DP.

As part of an ongoing investigation into the properties of the novel 5-HT_1-like receptor agonist GR43175 (Humphrey et al, 1988), we have examined its effect at 5-HT_{1D} binding sites.

The brains of male piglets (Large White variety, 3-12 days old, weighing 1-5kg) were removed and the caudate nuclei used to prepare a crude membrane fraction for binding assays as described by Heuring and Peroutka (1987). The assays contained 8-hydroxy-DPAT (100nM) and mesulergine (100nM) to prevent labelling of 5-HT_{1A} and 5-HT_{1C} sites respectively. Specific ^{3}H-5-hydroxytryptamine (^{3}H-5-HT) binding was defined as that inhibited by 10μM unlabelled 5-HT and represented 40-60% of total ^{3}H-5-HT binding. Competition curves were analysed using the programmes ALLFIT (DeLean, et al, 1978) and LIGAND (Munson and Rodbard, 1980). Results are means ± s.e.m. of at least three separate determinations.

Under the conditions employed, ^{3}H-5-HT apparently labelled a homogeneous population of sites (Kd 19±4nM, Bmax 0.36±0.03nM, Hill coefficient 0.98 ±0.01). The following compounds displayed little or no affinity (Ki values >1000nM) for these sites: spiperone, 8-hydroxy-DPAT, mesulergine, ketanserin, GR38032 and cyanopindolol.

Unlabelled 5-HT produced a monophasic inhibition of specific ^{3}H-5-HT binding (Ki 16 ±5nM, slope 0.95 ± 0.15), but 5-carboxamidotryptamine (5-CT) appeared to produce a biphasic inhibition curve (slope 0.31 ± 0.24) which, albeit on the basis of limited data points, could be fitted to a two-site model described by Ki values of 1.1 ± 0.4nM and 2.5 ± 1.7μM, representing 48 ± 4% and 52 ± 4% respectively of the total number of specific ^{3}H-5-HT binding sites.

GR43175 produced a monophasic inhibition (Ki 251 ± 103nM, slope 0.98 ± 0.17), but the curve consistently plateaued at 52 ± 8% inhibition of specific ^{3}H-5-HT binding when compared to 100% for 5-HT or 5-CT. In the presence of a maximally effective concentration of GR43175 (10μM), 5-CT produced a monophasic inhibition curve (slope 0.89 ± 0.20) with a Ki value of 0.8 ± 0.71μM.

These data are consistent with suggestions that 5-HT_{1D} sites are heterogeneous (Waeber et al, 1988), and we would speculate that the sub-site which recognises GR43175 and displays high affinity for 5-CT may correspond to the 5-HT_1-like receptor in the dog saphenous vein (Feniuk et al, 1985 and Humphrey et al, 1988).

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BEHAVIOURAL EVIDENCE FOR AN INTERACTION BETWEEN 5-HT_2 AND 5-HT_{1A} RECEPTOR FUNCTION

L.I.Backus*, T. Sharp & D.G. Grahame-Smith, MRC Unit & University Department of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, Oxford OX2 6HE

Recent electrophysiological, neuroendocrine and behavioural studies have highlighted the possibility of an interaction between central 5-HT_2 and 5-HT_1 receptor function (see Charig et al., 1986). In this study we investigated the effect of ritanserin, a selective 5-HT_2 antagonist, on the behavioural syndrome induced by the centrally acting 5-HT_{1A} agonists 8-OH-DPAT, 5-MeODMT, and gepirone in intact rats and on the contralateral rotation induced by 8-OH-DPAT in rats with unilateral lesions of forebrain 5-HT pathways.

Male adult rats (200-250g), were scored on individual components of the syndrome (head weaving, forepaw treading, hind limb abduction, flat body posture) on a 4-point ranked intensity scale (0=absent, 1=equivocal, 2=definite, 3=extreme) during observation periods of 45 seconds per rat. For 8-OH-DPAT and gepirone, the 6 observations began 10 minutes after injection and continued at 10 minute intervals for 1hr. For 5-MeODMT, the 6 observation periods began 5 minutes after injection and continued at 5 minute intervals for 30 min. The method for unilateral 5-HT lesions and the measurement of rotation was the same as described previously (Backus et al.,1988). Ritanserin or vehicle was administered 30 min before the 5-HT_{1A} ligands.

Ritanserin (0.1-2 mg/kg s.c.) dose dependently increased all four components and the total score (Table 1) of the 5-HT behavioural syndrome induced by 0.75 mg/kg 8-OH-DPAT. The dose response curve of the 8-OH-DPAT (0.1-3 mg/kg s.c.) behavioural syndrome components and total score were shifted to the left by ritanserin (0.2 mg/kg s.c.). Ritanserin (0.1-0.4 mg/kg s.c.) also dose-dependently increased the total score (Table 1) and 4 component scores for the 5-MeODMT-induced (2 mg/kg s.c.) behavioural syndrome. Finally, a single dose of ritanserin (0.2 mg/kg) significantly increased the gepirone-induced (10 mg/kg s.c.) behavioural syndrome (Table 1). The enhancement by ritanserin of the 8-OH-DPAT-induced behavioural syndrome was also observed in rats with whole brain 5-HT depletions produced by icv injection of 5,7-DHT (median total score vehicle+8-OH-DPAT 26.5 [-1.5, +1.5], ritanserin 0.4 mg/kg+8-OH-DPAT 46* [-3, +2] *p<0.01 Mann Whitney U. In rats with unilateral lesions of the medial forebrain bundle, median 8-OH-DPAT-induced (1 mg/kg s.c.) contralateral rotation was increased from 41.5 [-7.5, +30.5] (n=16), to 73.5* [-27.5, +28.5] (n=6) with ritanserin 0.1 mg/kg s.c. and to 95.5** [-49.5, +19.5] (n=10) with ritanserin 0.2 mg/kg s.c.(*p<0.05, **p<0.01 Wilcoxon signed rank test).

Table 1. TOTAL behaviour

Ritanserin (mg/kg s.c.)	% Baseline mean±s.e.m. (n)
8-OH-DPAT-induced behaviour	
0	100 ± 2.3 (24)
0.1	132* ± 9.2 (6)
0.2	150* ± 9.5 (6)
0.4	165* ± 4.3 (6)
2	169* ± 4.6 (6)
5-MeODMT-induced behaviour	
0	100 ± 14.0 (18)
0.1	169* ± 5.3 (6)
0.2	212* ± 7.6 (6)
0.4	222* ± 6.7 (6)
Gepirone-induced behaviour	
0	100 ± 7.2 (6)
0.2	158* ± 10.2 (6)

*p<0.01 Mann Whitney against concurrently tested controls.

One interpretation of these results is that 5-HT_2 function inhibits post-synaptic 5-HT_{1A} receptor function in the rat brain.

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MODULATION BY GTP OF THE INHIBITION OF [^{3}H]-DOB BINDING

Martyn D. Wood*, Beverley S. Alexander and Yvonne Reilly, Wyeth Research, Huntercombe Lane South, Maidenhead, Berkshire. SL6 OPH. U.K.

DOB ((±)-4-bromo-2,5-dimethoxyphenylisopropylamine) acts as an agonist at 5-HT$_2$ receptors (Glennon et al., 1983; Heller and Baraben, 1987). Binding studies with radiolabelled DOB have suggested on the one hand that [^{3}H]-DOB labels an agonist state of the 5-HT$_2$ receptor (Lyon et al., 1987) and on the other that (^{77}Br)-DOB labels a subset of 5-HT$_2$ recognition sites (Peroutka et al., 1988). GTP inhibits binding of both ligands and reduces agonist, but not antagonist, affinity for [^{3}H]-ketanserin-labelled 5-HT$_2$ receptors (Shearman and Strange, 1988). It was therefore of interest to determine whether GTP modulates agonist potency for [^{3}H]-DOB binding sites.

[^{3}H]-DOB (0.4nM) and [^{3}H]-ketanserin (1nM) were incubated in a final volume of 1ml with membranes from rat frontal cortex (18 mg and 9 mg original tissue respectively) at 37°C for 20 min and 30 min respectively. $MgCl_2$ (10mM) was included in the [^{3}H]-DOB assay. Specific binding was defined by 10^{-5}M mianserin.

In saturation studies, the Bmax for [^{3}H]-DOB binding was markedly lower than that for [^{3}H]-ketanserin (56.1 ± 7.6 and 197 ± 17 fmol/mg protein respectively, n = 3). Antagonists such as spiperone and mianserin were generally more potent displacers of [^{3}H]-ketanserin binding (IC_{50}'s 0.84 ± 0.31nM and 6.8 ± 1.8nM respectively) than of [^{3}H]-DOB binding (IC_{50}'s 2.13 ± 0.05nM and 27.2 ± 3.0nM respectively). Conversely 'agonists' such as 5-HT and the iodo analogue of DOB, DOI, were more potent displacers of [^{3}H]-DOB binding (IC_{50}'s 24.2 ± 6.6nM and 2.18 ± 0.21nM respectively) than of [^{3}H]-ketanserin binding (IC_{50}'s 2,200 ± 24nM and 46.3 ± 12nM respectively).

The piperazines MK 212 and 1-naphthylpiperazine (1-NP) show agonist and antagonist activities respectively at the central 5-HT$_2$ receptor in vitro. GTP (1mM final) significantly reduced the potency (IC_{50}) of MK 212 from 499 ± 65nM to 1140 ± 230nM ($p < 0.05$, Student's paired t-test) for [^{3}H]-DOB binding, but had no significant effect on that for 1-NP (39.1 ± 16nM control, 42.6 ± 5.9nM + GTP). GTP also reduced the potency of TFMPP against [^{3}H]-DOB from 24.2 ± 3.2nM to 54.0 ± 6.7nM ($p < 0.05$, Student's paired t-test). TFMPP displayed low affinity for [^{3}H]-ketanserin binding with an IC_{50} of 1570 ± 620nM. Interpretation of GTP shifts is complicated when using an agonist radioligand as GTP will reduce this binding. However, the profile obtained with the agonist MK 212 and the antagonist 1-NP are predicted using this model. The shift obtained with TFMPP, an antagonist at the 5-HT$_2$ receptor (Conn and Sanders-Bush, 1987) is therefore not consistent with [^{3}H]-DOB labelling the 5-HT$_2$ receptor.

These results are consistent with the suggestion that [^{3}H]-DOB labels an agonist site which is coupled to a guanine nucleotide regulatory protein. Whether this site represents an agonist state of the 5-HT$_2$ receptor or a receptor subtype remains to be determined.

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BACK MUSCLE CONTRACTIONS EVOKED BY SPINAL 5-HT_2 RECEPTOR ACTIVATION ARE POTENTIATED BY A TRH ANALOGUE (RX 77368)

K.C.F. Fone*, G.W. Bennett & C.A. Marsden, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Bulbospinal raphe neurones (BRN) which synapse with motoneurones contain both 5-hydroxytryptamine (5-HT) and thyrotrophin-releasing hormone (TRH), but whether 5-HT and TRH interact in the control of spinal motor function is unknown. We recently proposed (Fone et al., 1989) that the motor behaviour, back muscle contractions (BMC), produced by intrathecal 5-HT agonist injection involved activation of spinal 5-HT_2 receptors. The present study re-examines the 5-HT receptor subtype involved in BMC in light of the similar affinity of many 5-HT drugs for both 5-HT_{1C} and 5-HT_2 receptors (Hoyer, 1988) and also examines the effect of a TRH analogue pGlu-His-3,3'-dimethyl-ProNH2 (RX 77368) on 5-HT agonist-induced BMC.

Male Wistar rats (270-320g) were anaesthetised with sodium methohexitone (60mg/kg i.p.) to implant an intrathecal cannula (Fone et al., 1989). After seven days recovery, two separate groups of rats received intrathecal injections, at four day intervals, of saline and either 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI; 1, 2, 10, 25 and 50 µg; n=6) which has high affinity for 5-HT_2 and 5-HT_{1C} receptors or 1-(3-chlorophenyl) piperazine (mCPP; 10, 25, 50 and 100 µg; n=5) a relatively more selective 5-HT_{1C} than 5-HT_2 receptor agonist (Kennett & Curzon, 1988). Four days after each dose-response study DOI (10 µg) or mCPP (50 µg) were given again 30 min after the selective 5-HT_2 receptor antagonist ketanserin (0.16 mg/kg i.p.). A third group of rats (n=5) received intrathecal injections of DOI (5 µg), alone (day 7), 30 min after RX 77368 (10 ng, day 15) and at the same time as RX 77368 (10 ng, day 19) and RX 77368 alone (10 ng, day 11). In all groups, the number of BMC were counted separately for 30 min following each injection and results are presented as mean $\pm$ s. e. mean.

The BMC produced by intrathecal DOI were significantly greater ($P<0.05$, ANOVAR) than observed with saline (2 $\pm$ 1) and increased linearly with $\log_{10}$ dose ($P<0.001$, method of least squares) from 18 $\pm$ 6 BMC in 30 min with 1 µg up to 173 $\pm$ 19 with 25 µg (with no further increase with 50 µg). Although mCPP produced significantly more BMC (16 $\pm$ 2 and 14 $\pm$ 3 BMC in 30 min with 10 and 100 µg, respectively) than saline ($P<0.05$, ANOVAR) the behaviour did not increase with dose. The BMC produced by both DOI and mCPP were significantly attenuated ($P<0.01$ Students' t-test) by ketanserin. Intrathecal pretreatment with RX 77368, which produced few BMC (8 $\pm$ 3 in 30 min) when given alone, significantly augmented the number of BMC (118 $\pm$ 14 compared with 54 $\pm$ 12, $P<0.05$, ANOVAR) induced by DOI (5 µg). In contrast, the number of BMCs (85 $\pm$ 17) observed when RX 77368 and DOI were given at the same time was not greater than the response obtained when these drugs were given individually.

The present data supports our previous evidence (Fone et al., 1989) that 5-HT-induced BMC are mediated by 5-HT_2 and not 5-HT_{1C} receptor activation. Since dose-related BMC were evoked by intrathecal injection of DOI, but not by the 5-HT_1 receptor agonist mCPP. Furthermore pretreatment with the 5-HT_2 receptor antagonist ketanserin reduced both DOI- and mCPP-induced BMC. The augmentation of 5-HT agonist-induced BMC by intrathecal TRH analogue pretreatment may be a functional consequence of the co-existence of 5-HT and TRH in BRN which innervate motoneurones.

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THE 5-HT_3 RECEPTOR LIGAND, [^{3}H]-BRL 43694, BINDS TO PRESYNAPTIC SITES IN THE NUCLEUS TRACTUS SOLITARIUS OF THE RAT

G.D. Pratt* & N.G. Bowery. Dept of Pharmacology, The School of Pharmacy, University of London, 29/39 Brunswick Square, London WC1N 1AX.

Binding of the 5-HT_3 receptor antagonist, [^{3}H]-BRL 43694 has been demonstrated in rat cortical membranes by Nelson and Thomas (Biochem. Pharm., in press). The unlabelled ligand has also been shown to be a potent displacer of [^{3}H]-GR 65630 (another 5-HT_3 antagonist) binding to homogenates of both rat entorhinal cortex and area postrema (AP, the location of the chemoreceptor trigger zone, involved in the coordination of emesis). (Kilpatrick et al., 1987, 1988a). Both ligands markedly reduce cisplatin-induced emesis in ferrets (Bermudez et al., 1988; Kilpatrick et al., 1988a). It is believed that cytotoxic therapy stimulates the release of neuroactive agents, notably 5-HT, from the gastrointestinal tract which activates gastrointestinal afferents particularly of the vagus nerve. These axons project to the AP and also to the nucleus tractus solitarius (NTS) the latter of which, together with the parvicellular reticular formation, visceral and somatic motor nuclei, constitute the vomitting centre. This functional consequence of 5-HT_3 receptor activation has thus prompted considerable interest into attempting to relate the anti-emetic action of these ligands with possible central binding sites in regions known to be associated with the emetic response. In view of this, Kilpatrick et al. (1988b) have found using autoradiographical analysis that in the rat hindbrain, [^{3}H]-GR 65630 binds solely to a high density of recognition sites located in the AP. We have now examined the autoradiography of [^{3}H]-BRL 43694 binding also in rat brain.

Brain sections (10 μm) from male Wistar rats were incubated at room temperature for 30 min. in 5mM HEPES buffer (pH 7.4) containing 10nM [^{3}H]-BRL 43694 (21 Ci/mmol). Non-specific binding was defined in the presence of 100uM GR 38032F. Binding was also examined in brain sections prepared 10 days after unilateral nodose ganglionectomy with or without chronic hemisection of the NTS. Autoradiograms were compared with adjacent sections stained with cresyl fast violet to define the location of binding sites.

Quantitative autoradiographical analysis revealed the highest specific binding of [^{3}H]-BRL 43694 to be in the NTS (495±32 fmol/mg tissue, mean ±sem of triplicate determinations from 5 rats) whereas binding to the AP represented up to 20% of that seen in the NTS. Binding was reduced unilaterally after nodose ganglionectomy by 45% in the caudal regions of the NTS to 90% in more rostral sections (7 sections). Additional hemisection of the NTS produced a further reduction in binding density.

In conclusion, we have shown that [^{3}H]-BRL 43694 binds to a high density of recognition sites in the NTS and that the reduction in binding following ganglionectomy would be indicative of a presynaptic receptor location on vagal afferent terminals.

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(+)-TUBOCURARINE POTENTLY ANTAGONISES 5-HT_3 RECEPTOR MEDIATED CURRENTS IN A NEURONAL CELL LINE (N1E-115)

John A. Peters* & Jeremy J. Lambert, Department of Pharmacology and Clinical Pharmacology, Ninewells Hospital and Medical School, DUNDEE. DD1 9SY.

In clonal N1E-115 neuroblastoma cells and some other neuronal cell lines such as NG 108-15 and NCB-20, 5-HT evokes an increase in membrane conductance, resulting in depolarizing or inward current responses that may be recorded under current- and voltage- clamp conditions respectively (Peters and Usherwood, 1983; Hales et al., 1988; Neijt et al., 1988; Yakel and Jackson, 1988). Although these effects are mediated by the 5-HT_3 receptor subtype, it has been observed that both the 5-HT-evoked depolarization and inward current are susceptible to blockade by (+)-tubocurarine ((+)-Tc) at unusually low concentrations (Peters and Usherwood, 1983; Yakel and Jackson, 1988). In the present study, the influence of (+)-Tc and 3 other nicotinic receptor antagonists upon 5-HT-evoked currents has been examined in voltage-clamp recordings from N1E-115 cells.

Cells were cultured and used in electrophysiological experiments as previously described (Peters et al., 1988). Inward currents evoked by ionophoretically applied 5-HT, recorded at a holding potential of -60 mV, were potently antagonised by bath applied (+)-Tc (0.3 - 10.0 nM). Blockade by (+)-Tc was rapid in onset, completely reversible on washout, and occurred with an IC_{50} of 0.85 nM, a value close to that of 0.74 nM previously reported in NG 108-15 cells (Yakel and Jackson, 1988). In comparison, the selective 5-HT_3 receptor antagonist GR 38032F (Butler et al., 1988) antagonised the response with an IC_{50} of approximately 0.2 nM. The depth of block produced by (+)-Tc (1 nM) was not dependent upon membrane potential over the range -80 to +20 mV, and the reversal potential of the 5-HT-evoked current recorded under control conditions (mean ± S.E. = -0.7 ± 0.3 mV; n=3) was similar to that observed in the presence of the drug (-0.8 ± 0.4 mV; n=3). (+)-Tc (3 nM) exhibited no obvious agonist-dependency in its blocking action. In contrast to (+)-Tc, the nicotinic receptor antagonists gallamine, vecuronium and trimetaphan, each applied at a concentration of 1 μM to 3 N1E-115 cells, produced little (<5%) or no block of the 5-HT-induced current.

In conclusion, the present study confirms that (+)-Tc potently antagonises 5-HT_3 receptor mediated responses in neuronal cell lines (Yakel and Jackson, 1988), and also suggests that such blockade is not use-dependent, voltage-dependent, or a general feature of nicotinic antagonists.

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LOCAL CEREBRAL GLUCOSE UTILISATION IN THE CONSCIOUS RAT AFTER GR38032F

E.A. Mitchell*, B.J. Jones+ & J.A. Pratt, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW & +Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 ODJ.

The $5HT_3$ antagonist GR38032F is anxiolytic in behavioural tests putatively through an action at $5HT_3$ receptors (Jones et al 1988). However, the neuroanatomical pathways which are functionally affected as a consequence of receptor occupation have not been identified. We have therefore used ^{14}C-2-deoxyglucose quantitative autoradiography (Sokoloff et al, 1977) to determine function related changes in cerebral glucose use after acute administration of GR38032F in conscious male PVG hooded rats.

Measurements were initiated 35 min following GR38032F (0.01-1.0 mg/kg ip) and glucose use determined in 66 brain areas. GR38032F effected alterations in only 13 of the 66 brain areas analysed: these included limbic, motor and cortical structures. In the majority of these 13 regions GR38032F (0.01 mg/kg) reduced cerebral glucose use compared to control values but this effect was not apparent following the larger doses (0.1 and 1.0 mg/kg). In several structures GR38032F (1.0 mg/kg) raised cerebral glucose use relative to the smaller dose (0.1 mg/kg) possible suggesting opposing actions of the drug at the different dosage levels (Table 1). Changes in glucose use did not always reflect areas where $5HT_3$ receptor density was highest. Thus, although glucose use was reduced in hippocampal layers it was unchanged in the entorhinal cortex and area postrema (Table 1).

Table 1 The effect of GR38032F on cerebral glucose utilization

		GR38032F (mg/kg)		
Structure	Control	0.01	0.10	1.0
Auditory Cortex (layer IV)	126±7	101±6*	129±8+	123±6+
Visual Cortex (layer IV)	89±3	72±2*	82±7	87±4+
Entorhinal Cortex	54±2	49±2	52±2	52±3
Median Raphe	83±2	81±3	86±4	95±2+
Hippocampus (CA_3 oriens)	67±2	54±2*	66±4+	65±3+
Hippocampus (CA_3 radiatum)	62±2	50±2*	63±4+	63±3+
Anteromedial Thalamic Nucleus	104±5	90±4	106±6	114±4+
Area Postrema	62±4	62±3	67±4	66±5

Data are presented as mean glucose use (μ mol/100g/min) ±SEM (n=6-7 per group). * $p<0.05$ compared to control, + $p<0.05$ compared to 0.01 mg/kg Analysis of variance followed by Newman Keul's multiple range test.

These data suggest that GR38032F has a more restricted action on cerebral function than the benzodiazepine anxiolytics which produce widespread reductions in glucose use (Laurie & Pratt, 1988). The changes effected in the hippocampus may reflect an altered neuronal activity within the hippocampus or be a result of changes in the activity of projection pathways to this area.

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DIFFERENCES IN THE CALCIUM SENSITIVITY OF INDIRECT cAMP RESPONSES TO 5-HT, NORADRENALINE AND GLUTAMATE IN GUINEA-PIG BRAIN

J. Donaldson* & S.J. Hill, Department of Physiology & Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH.

Neurotransmitters can modify cAMP levels in brain slices either directly (via eg. adenosine A_2-, histamine H2- or β-adrenergic-receptors) or indirectly (via eg. α_1-adrenergic-, histamine H1- or 5-hydroxytryptamine (5HT) receptors) (Hill & Kendall, 1989). The indirect mechanism involves the augmentation of the cAMP response to a directly acting neurotransmitter. We have recently presented evidence for a role for calcium in the H_1-receptor mediated augmentation of the 2-chloroadenosine (2CA) cAMP response in guinea-pig cerebral cortex (Donaldson et al, 1988). However, data presented by Kendall et al (1989) have suggested that the mechanism of glutamate-mediated augmentation of 2CA in the same tissue differs from that of histamine, since the glutamate, but not the histamine response was blocked by forskolin. We have therefore examined the calcium sensitivity of a number of other indirect cAMP responses in guinea-pig cerebral cortex.

cAMP accumulation was measured in slices of guinea-pig cerebral cortex labelled with ^{3}H-adenine and incubated with magnetic stirring in Krebs-Henseleit solution containing 2.5mM $CaCl_2$ as described previously (Donaldson et al, 1987). The stable adenosine analogue 2CA was used as the direct cAMP stimulus. The effects of endogenous adenosine were eliminated with adenosine deaminase (1.2 U/ml). Once a steady level of cAMP had been achieved in the presence of 2CA (30μM), 5HT (0.3mM), glutamate (3mM) or noradrenaline (NA, 0.1mM) were added. Extracellular calcium was rapidly reduced to below 0.1uM by addition of EGTA (5mM, final pH 7.4) 2 min before agonist addition at 2CA steady state.

Addition of EGTA at 2CA steady state produced a small increase in the cAMP level (1.35 ± 0.08 fold, n=12), but greatly attenuated the increase in cAMP produced by subsequent addition of 5HT or NA. The peak of the increase in cAMP accumulation following addition of 5HT or NA in the presence of EGTA, was reduced by 64.2 ± 6.9%(n=5, for 5HT) or 60.9 ± 1.6%(n=3, for NA) of that measured in the absence of EGTA. The extent of the reduction of 5HT and NA responses in the presence of EGTA was similar to that found previously for histamine (Donaldson et al, 1988). The glutamate response, however, was much less sensitive to the EGTA-induced reduction in extracellular calcium, being reduced by only 11.8 ± 3.4 % (n=10) in the presence of EGTA. In contrast, the glutamate response was completely abolished by addition of 1μM forskolin 2 min before glutamate at 2CA steady state (n=3), whilst the histamine response was reduced by only 15.7 ± 7.5 % (n=3) in the presence of forskolin.

These results therefore suggest that calcium has a role in the augmentation of the 2CA cAMP response produced by NA and 5HT, as well as histamine, but that calcium is much less important in the glutamate response. These data therefore provide further evidence that there is more than one mechanism by which the augmentation of cAMP responses can occur.

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THE [^{3}H]-LEUKOTRIENE D_4 BINDING SITE IN GUINEA-PIG LUNG CORRELATES WITH A LEUKOTRIENE RECEPTOR ON GUINEA-PIG ILEUM

P. Norman, T.S. Abram, N.J. Cuthbert and P.J. Gardiner, Bayer Research, Stoke Court, Stoke Poges, Slough. SL2 4LY.

[^{3}H]leukotriene (LT) D_4 binding to guinea-pig lung membranes has previously been shown to be of high affinity, saturable and sterospecific (Pong and De Haven, 1983. Norman et al, 1984). We have also reported that guinea-pig ileum contains two types of leukotriene receptor (Cuthbert and Gardiner, 1987). Having synthesised a number of structural derivatives of LTD_4 and LTE_4 we investigated their functional activity on guinea-pig ileum and their ability to inhibit 0.8nM [^{3}H]-LTD_4 binding to guinea-pig lung membranes.

All the compounds had spasmogenic activity on longitudinal strips of guinea-pig ileum and over the concentration ranges tested (10^{-9} - 10^{-5}M) were found to produce maximal responses 50 - 100% of the LTD_4 maximum. The EC_{50} values were calculated from the cumulative concentration-response curves and are shown in Table 1 as their negative logarithms (pD_2).

Table 1 Leukotriene binding affinity to guinea-pig lung membranes (n > 3) and contractile activity on guinea pig ileum (n > 3)

Leukotriene	pKi	pD_2	Leukotriene	pKi	pD_2
LTD_4	8.97	8.40	LTE_4	8.69	7.92
7(E) 9(Z) LTD_2	8.84	8.60	D-LTE_4	8.70	8.60
7(Z)-LTD_1	8.08	7.20	Deamino LTE_4	8.77	8.58
LTD_0	6.47	6.68	LTF_4	6.42	6.15
18,19,20 trinor LTD_3	6.02	5.63	C-glu LTE_4	5.75	5.74
14-20 heptanor LTD_3	4.46	4.50	1-decarboxy 1,2,3,4 tetranor LTD_4	5.23	5.72
D-LTD_4	7.90	7.84			
N-Acetyl LTD_4	7.73	7.35			

All compounds completely inhibited specific binding, defined as binding of [^{3}H] LTD_4 displaceable by 2μm LTD_4, IC_{50} values were calculated by log-logit transformation and converted to Ki values, also tabulated as negative logarithms. Least squares linear regression indicated the pKi and pD_2 values to be significantly correlated (slope 0.905 ± 0.006, r = 0.972, P < 0.001).

The highly significant correlation between the binding affinity and agonist activity indicates that the [^{3}H]-LTD_4 binding site in guinea-pig lung is similar to the leukotriene receptor, preferentially activated by LTD_4 and LTE_4, present on guinea-pig ileum. Furthermore since the binding affinity correlates directly with the pD_2 values it is apparent that all these agonists must have low efficacy at this receptor on the ileum.

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INHIBITION OF 5-LIPOXYGENASE BY SL 81.0433

C.N. Berry*, D.C. Chastagnol-Hermann & K.G. Lloyd. SYNTHELABO RECHERCHE (L.E.R.S.), 23-25 Ave Morane Saulnier, 92366 Meudon-La-Forêt Cédex, France.

We have shown (Prouteau et al, 1989) that SL 81.0433 (4[[(5-chloro-2-hydroxy-3 propylphenyl)(4-chloro-phenyl)methylene]amino]butanamide) is active against four different models of gastric ulceration in the rat. Given that it has been proposed that leukotrienes (LTs) particularly LTC_4 may play a role in the pathogenesis of certain models of gastric ulceration (Peskar et al, 1986, inter alia), it was decided to examine the effects of SL 81.0433 on 5-lipoxygenase in vitro and on the increase in gastric mucosal LTC_4 formation in ethanol-induced ulcers ex vivo at its ED_{50} in this model.

Cells obtained by lavage of rat (male Sprague-Dawley, 200-250 g) peritoneal cavities were preincubated for 10 min at 37°C in phosphate buffered saline containing Ca^{++}, Mg^{++} and GSH (3mM) with the test compounds or their vehicles before the addition of arachidonic acid (10 µg/ml) and a further incubation for 10 min. After stopping the reactions by centrifugation (400 g, 10 min, 37°C), the supernatants were assayed for LTB_4 using Amersham RIA kits. Fragments of gastric mucosa (50-100 mg, wet weight) taken from female Wistar rats (200-220 g) were incubated in Tyrode's solution (1 ml) with the drug or vehicle, 3 mM GSH and 50µl Na_2CO_3 (0.5%) at 37°C for 30 min, after a 30 min preincubation at 4°C (also in 1 ml Tyrode's solution) with the drug. The fragments were removed and dried and the peptido LTs were assayed, after boiling the incubates for 2 min, with $LTC_4/D_4/E_4$ RIA kits (Amersham). IC_{50} values were estimated from three-point log-dose-inhibition curves from at least three separate experiments. In the ex vivo experiments, fasted female Wistar rats (200-220 g, 6 per group) received either drug or vehicle 30 min before oral injection of 50% ethanol (1 ml per rat). One hour later, the animals were killed and gastric mucosal peptido-LT synthesis was measured as above. Tests for statistical significance were performed using Student's t-test for unpaired samples.

The peritoneal cells (1.1-1.6 x 10^6 cells/ml) generated 10-16 ng/ml immunoreactive LTB_4 in the control samples. This synthesis was dose-dependently inhibited by SL 81.0433 with an estimated IC_{50} (mean ± s.e.m.) of 4.2 ± 0.8 µM (n=3) which was comparable with that obtained with BW755C used as a reference compound (IC_{50}= 3.3 ± 0.5 µM, n=7). SL 81.0433 also inhibited gastric mucosal petido-LT formation in vitro (mean IC_{50} ± s.e.m. = 12.4 ± 10.0 µM, n=3) once again with an activity comparable to BW755C (mean IC_{50}= 11.9 ± 10.0 µM, n=3). In the ex vivo experiments, control mucosal fragments released 270 ± 37 ng/g dry weight (mean ± s.e.m.) peptido-LT, whilst in rats receiving ethanol alone peptido-LT formation was increased to 515 ± 64 ng/g dry weight ($p<0.001$, n=6); at the same time there was marked gastric ulceration (mean ulcer score= 3.7 ± 0.2). SL 81.0433 pre-treatment (60 $mg.kg^{-1}$) significantly prevented lesion formation (ulcer score= 1.5 ± 0.3, n=6, $p<0.001$) while ex vivo mucosal peptido-LT formation had almost returned to control levels (304 ± 37 ng/g dry weight, $p<0.05$ vs ethanol alone).

These results suggest that SL 81.0433 may owe its "gastroprotective" properties at least in part to an inhibition of 5-lipoxygenase.

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Ca^{2+}-DRIVEN PROSTACYCLIN SYNTHESIS AND VON WILLEBRAND FACTOR SECRETION IN ELECTROPERMEABILISED ENDOTHELIAL CELLS

Michael C. Scrutton* & Jeremy D. Pearson. *Division of Biomolecular Sciences, King's College, Campden Hill Road, London W8 7AH and Section of Vascular Biology, MRC Clinical Research Centre, Harrow, Middx, HA1 3UJ.

Previous studies using intact human umbilical vein endothelial cells (HUVEC) indicate that an increase in the concentration of cytosolic Ca^{2+} ($[Ca^{2+}]_i$), can induce release of prostacyclin (PGI_2) and von Willebrand factor (vWf). Activation of protein kinase C by phorbol 12-myristate 13-acetate (PMA) induces vWf secretion and potentiates Ca^{++}-driven PGI_2 release (De Groot et al 1984; Demolle & Boeynaems 1988). Hence at least one other transduction pathway can modulate these responses. To define directly the dose/response relationships between $[Ca^{2+}]_i$ and secretion of PGI_2 and vWf, and to quantify the effect of other factors on these processes, we have established an electropermeabilised endothelial cell preparation in which the cytosolic environment can be manipulated systematically. HUVEC (passage 2 or 3) were grown to confluence in 16mm wells, washed with serum-free medium and then covered with electropermeabilisation medium (0.28M glycine/0.02M K glutamate/0.02M K PIPES/2mM Mg acetate/5mM EGTA pH 6.6). The confluent monolayer was permeabilised by 5 discharges of a $2.2KVcm^{-1}$ electric field ($\tau \approx 30$ s) applied using two plane stainless steel electrodes 10mm apart. After each discharge the electrode orientation was altered with respect to the cell monolayer. The extent and stability of permeabilisation was assessed by visual estimation of ethidium bromide uptake using fluorescence microscopy. Under the conditions used the permeabilised preparation was stable for >60 min and no significant increase in release of lactate dehydrogenase occurred. Additions (eg guanine nucleotides, PMA) were made immediately after application of the electric field. After 7-8 mins the permeabilisation medium was removed and replaced with a similar medium without additions but containing 2.4mM Mg ATP^{2-} and Ca^{2+} concentrations buffered in the range 0.05-30μM using a Ca/EGTA buffer system. PGI_2 release was measured by specific RIA (for 6-keto-$PGF_{1\alpha}$) and vWf secretion by specific ELISA.

After a lag of approximately 10 mins linear Ca^{2+}-dependent PGI_2 release is observed. The threshold $[Ca^{2+}]$ for induction of significant PGI_2 synthesis is 0.8-1.0μM. Addition of 20μM Ca^{2+} causes a 10-to-20-fold increase in the rate of PGI_2 release with an EC_{50} (Ca^{2+}) of 2.4±0.5μM (n = 10). In contrast significant vWf release occurs at very low (≈0.01μM) Ca^{2+}. Ca^{2+}-driven vWf release only occurs when $[Ca^{2+}]$ exceeds 2-3μM and the maximal extent of this response cannot be defined in our system.

Preincubation of electropermeabilised HUVEC with either PMA (10-20nM) or GTPγS (10-100μM) enhances the maximal extent and decreases the EC_{50} (Ca^{2+}) for Ca^{2+}-driven PGI_2 release but has no consistent effect on the properties of vWf release. In the presence of PMA or GTPγS the threshold $[Ca^{2+}]$ for PGI_2 release is 0.2-0.3μM and the EC_{50} (Ca^{2+}), 0.5-0.8μM.

Electropermeabilisation of attached endothelial cells provides a suitable preparation for assessment and manipulation of putative signal transduction pathways in these cells. The data presented define quantitatively the involvement of Ca^{2+} in PGI_2 and vWf release and indicate the activation of a guanine nucleotide-binding protein and/or protein kinase C modulates Ca^{2+}-driven PGI_2 release.

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EFFECT OF SC-19220 ON NEUROTRANSMISSION IN THE GUINEA-PIG ILEUM

Poll, C., *Grix, S., and Coleman, R.A., Department of Peripheral Pharmacology, Glaxo Group Research Ltd., Ware, Herts, SG12 0DJ.

Guinea-pig ileum (GPI) contains prostanoid receptors mediating both smooth muscle contraction and enhancement of cholinergic neurotransmission (Poll et al, 1988a). The prostanoid receptors mediating smooth muscle contraction are of the EP_1-type, being specifically blocked by both SC-19220 and AH6809 (Coleman et al, 1985). In contrast, the neuronal effects are unaffected by AH6809, suggesting that EP_1-receptors are not involved (Poll et al, 1988a). However, as SC-19220 has well documented neuronal blocking activity in a range of preparations (Bennett, 1974; Horrobin et al, 1977), we have now studied its effects in the GPI, both against responses to cholinergic nerve stimulation and against prostaglandin (PG)E_2-induced enhancement of those responses.

Longitudinal muscle strips of GPI, with myenteric plexus intact, were prepared and mounted in organ baths for electrical field stimulation, as previously described (Poll et al, 1988a). All experiments were carried out in the presence of indomethacin (3μM) and AH6809 (10μM).

SC-19220 (30 and 100μM, n=4) produced 16±4% and 40±13% inhibition of electrically-induced neuronal responses respectively, but had no effect on direct contractile responses to exogenous acetylcholine (1-3000nM, n=2). PGE_2 (0.1-300nM)-induced enhancement of responses to neuronal stimulation was inhibited by SC-19220 (30 and 100μM), yielding concentration-ratios (CR) of 2.8 (1.4-5.7, n=7) and 31.9 (7.9-122, n=4) respectively. Inhibition by SC-19220 (100μM) was also associated with a reduction in the maximum response to PGE_2 of 38±14%. 5-HT (0.1-300nM)-induced enhancement of neuronally-mediated responses was similarly inhibited by SC-19220 (30μM), yielding a CR of 4.4 (2.2-8.5, n=3) with a 49±10% reduction of the maximum response.

We have previously shown that cyclopentyl adenosine (CPA), like SC-19220, also inhibits neuronally-mediated responses of guinea-pig ileum. This inhibition was attenuated by pretreatment of guinea-pigs with pertussis toxin (PTX), implicating G_i regulatory protein in CPA activity (Poll et al., 1988b). However, PTX pretreatment has no effect on SC-19220 (100μM)-induced inhibition of neuronal responses (n=5).

Finally, the local anaesthetic agent procaine (100μM), like SC-19220, caused inhibition of both neuronally-mediated responses of GPI- and of PGE_2- and 5-HT-induced enhancement of such responses, with mean CRs of 69 (n=2) and >400 (n=2) respectively.

In conclusion, therefore, although SC-19220 is a specific prostanoid EP_1-receptor blocking drug, its inhibitory effect against neuronal responses in GPI, and its antagonism of PGE_2-induced enhancement of these responses do not result from specific prostanoid receptor blockade. The inhibitory actions of SC-19220 are presynaptic in nature, and do not appear to involve a "G_i-like" regulatory protein. SC-19220-induced neuronal inhibition in GPI may result from a 'procaine-like" local anaesthetic action.

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PROTEIN KINASE C ACTIVATION REDUCES THE INTRACELLULAR CALCIUM THRESHOLD FOR AGONIST-STIMULATED PROSTACYCLIN RELEASE

T.D. Carter*, T.J. Hallam[1] & J.D. Pearson (introduced by M.C. Scrutton), Section of Vascular Biology, MRC Clinical Research Centre, Harrow, Middx HA1 3UJ, [1]Dept. Cellular Pharmacology, Smith Kline & French Research, Welwyn, Herts AL6 9AR.

We have shown that agonist-stimulated prostacyclin (PGI_2) release from human umbilical vein endothelial cells (HUVEC) is normally driven by elevations of intracellular calcium, $[Ca^{++}]_i$, above a threshold of 0.8-1μM (Carter et al, 1988; Hallam et al, 1988). It has recently been found, however, that protein kinase C activation by phorbol 12-myristate 13-acetate (PMA) potentiates ionophore-induced PGI_2 release (Demolle & Boeynaems, 1988) but inhibits agonist-stimulated inositol lipid hydrolysis and $[Ca^{++}]_i$ elevations in endothelial cells (Brock & Capasso, 1988). We have now studied the effect of PMA on agonist-induced PGI_2 release and determined how it is related to changes in $[Ca^{++}]_i$. HUVEC were grown in 16mm wells of 24 well trays for the replicate determination of PGI_2 release (assayed by specific RIA for 6-keto-$PGF_{1\alpha}$). HUVEC grown on glass coverslips were loaded with the calcium sensitive fluorescent dye fura-2 for the concomitant measurement of $[Ca^{++}]_i$ and PGI_2 release. The PMA analogue 4α-phorbol 12,13-didecanoate (100nM) had no effect on basal or stimulated PGI_2 release, or $[Ca^{++}]_i$, in any experiment.

PMA alone enhanced the basal rate of PGI_2 release in a dose and time dependent manner, but with a lag period of >10 min even at the highest dose tested (100nM). Pretreatment with PMA caused a time and dose dependent potentiation of the transient PGI_2 release induced by submaximal doses of agonists (0.02U/ml thrombin, 10nM bradykinin, 0.5μM histamine, 10μM ATP). Maximum potentiation (up to 3.6-fold) was achieved within 5-10 min of pretreatment; threshold, EC_{50} and maximally active doses of PMA were 1, 5 and 50nM. The transience of the agonist-stimulated responses was unaltered. PMA did not potentiate PGI_2 release in response to added arachidonate (4μM).

PMA alone had no effect on resting $[Ca^{++}]_i$ (≈100nM) even at doses and times where it induced PGI_2 release. In the absence of PMA, ATP (1-100μM) dose-dependently increased $[Ca^{++}]_i$ and PGI_2 release. Pretreatment for 5 min with PMA (1-100nM) dose-dependently reduced peak $[Ca^{++}]_i$ in response to all tested concentrations of ATP, while PGI_2 release in response to 100 or 10μM ATP was potentiated and in response to 1μM ATP was unaffected. Similarly divergent effects of PMA pretreatment on $[Ca^{++}]_i$ and PGI_2 release were found with thrombin, histamine or bradykinin as agonists.

The effect of PMA on the Ca^{++} dependence of PGI_2 synthesis was determined directly by concomitant measurement of $[Ca^{++}]_i$ and PGI_2 release from cells exposed to graded doses of ionomycin in the absence of extracellular Ca^{++}. Pretreatment with PMA (5 min; 10nM) shifted the Ca^{++} activation curve to the left and decreased the threshold $[Ca^{++}]_i$ from 0.8-1μM to 0.2-0.3μM.

We conclude that activation of protein kinase C has two consequences that affect PGI_2 release. First, it reduces the peak $[Ca^{++}]_i$ achieved in response to agonists, by reducing Ca^{++} mobilisation and/or increasing Ca^{++} sequestration or extrusion. Second, probably by an effect on phospholipase A_2, it enhances the sensitivity of PGI_2 synthesis to $[Ca^{++}]_i$. The interaction of these two processes is sufficient to account quantitatively for the observed effects of PMA on basal and agonist-stimulated PGI_2 synthesis.

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THE CAUSE OF REGIONAL DIFFERENCES IN PROSTACYCLIN PRODUCTION BY PREGNANT RAT MYOMETRIUM

Linda A. Harrison, R.J. Flower and K.I. Williams*
Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.

Rat pregnant myometrium synthesises large quantitites of prostacyclin (PGI_2) (El Tahir and Williams, 1979). However production is not uniform as the areas of myometrium in contact with the placenta (MUP) produce negligible PGI_2 compared with the remainder of the myometrium (M) (Williams and El Tahir, 1980). We have now carried out experiments to investigate the causes of this differential production.

Female Wistar rats were killed on days 20-21 of pregnancy, uterine horns dissected out and areas of myometrium beneath the placenta separated from the remaining myometrium. Weighed samples of M and MUP were suspended in 1 ml of ice-cold Tris buffer 0.05M pH 8, chopped finely and centrifuged at 4750g for 2 min. Samples were then resuspended to 25% ($^{w}/v$) and incubated for 15 min. at 20°C. Aliquots of incubation medium were stored on ice until assayed for their anti-aggregatory activities against authentic PGI_2 using citrated rabbit platelet-rich plasma (PRP) (El Tahir and Williams, 1979).

M produced 3.94 ± 0.39 ng/mg compared to 0.08 ± 0.01 (mean ± SEM. n=3) ng/mg by MUP. Arachidonic acid caused a dose-dependent increase in PGI_2 production, at 32.8 μM a 115% increase over basal production by M was seen, whereas in MUP a 58% increase was noted.

A series of experiments were then carried out to determine whether increased catabolism contributed to these effects. Samples of M and MUP were homogenised in Tris buffer plus EDTA 1 mM (to inhibit phospholipase A_2 activation). The homogenates were centrifuged at 1500g for 15 min and the supernatants incubated with 0.5μCi ^{14}C-AA; subsequent microsomal pellets (110,000g 60 min) were incubated similarly. After acidic lipid extraction products were separated by thin-layer chromatography using the organic phase from ethylacetate, iso-octane, acetic acid, water (11:5:2:10). Autoradiograms were prepared prior to assessment of isotope contents of different regions of the plates with R_f values corresponding to authentic prostaglandins. The major product of M and MUP in both the low-speed and microsomal fractions was 6-oxo-$F_{1\alpha}$. For MUP synthesis was similar in both fractions (microsomal fraction excludes PG catabolising enzymes). Furthermore incubation of M microsomes in MUP microsomal supernatant did not decrease 6-oxo-$F_{1\alpha}$ production. We conclude that low PGI_2 production by MUP is not due to increased catabolism or a shift to synthesis of other PGs. The cause appears to be depressed cyclo-oxygenase and/or prostacyclin synthetase activities. The factor(s) responsible for this effect are currently under study.

L.A.H. is a SERC student

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THE RELEASE OF AN EPITHELIUM DERIVED INHIBITORY FACTOR FROM RABBIT INTRAPULMONARY BRONCHI

D. Spina* and C.P. Page. Department of Pharmacology, King's College, University of London, Manresa Road SW3 6LX.

Considerable interest has been generated regarding the contribution of epithelial cell damage to non-specific bronchial hyperreactivity in asthma, since epithelial cell damage is observed in asthma (Laitinen et al., 1985). Many studies have documented that removal of the epithelium results in enhanced smooth muscle responsiveness to various spasmogens in vitro (for rev see Fedan et al., 1988). Such studies provide only indirect evidence for the existence of an epithelial derived relaxant factor. Recently, a co-axial bioassay system has been used to demonstrate the transfer of an inhibitory factor from guinea-pig tracheal epithelial cells (Ilhan & Sahin, 1986; Fernandes et al., 1989). We have used a similar technique to determine whether epithelial cells from rabbit intrapulmonary bronchi release an inhibitory factor.

Aorta from male Wistar rats (200-300 g) were denuded of endothelium using a cotton wool swab and cut into zig-zag strips and suspended in an organ bath containing Krebs-Henseleit solution aerated with 95% O_2 and 5% CO_2 at 37°C under 500 mg tension. Endothelium-denuded rat aorta contracted with (-)-phenylephrine (0.05 uM) failed to relax in response to methacholine (100 uM), while (+)-isoprenaline (1 uM) caused a 93 ± 4 % (n = 8 animals) reduction of the phenylephrine-induced contractile response.

White New Zealand rabbits (1.8-3.0 kg) of either sex, were anaesthetized with Valium (5 mg/kg) and Hypnorm (0.4 ml/kg), then exanguinated. Intrapulmonary bronchial tubes (1 cm) which were free of alveolar tissue and visible blood vessels, were placed over the rat aorta and allowed to equilibrate for 45 minutes. Rat aorta which were contracted with (-)-phenylephrine (10 uM), relaxed in a concentration-dependent manner to methacholine, yielding a pD_2 ($-\log_{10} EC_{50}$) value of -5.566 ± 0.066 (n = 8 animals). Atropine (0.03-0.1 uM), produced a concentration-dependent antagonism of methacholine-induced relaxation of the rat aorta. Schild regression to atropine gave a pA_2 value of 8.86 ± 0.16 and slope of 1.13 ± 0.08 (n = 3 animals). Incubation of the co-axial bioassay preparation for 30 min with indomethacin (5 uM) or methylene blue (10 uM) failed to alter the relaxant potency of methacholine ($P > 0.05$, paired t-test). In contrast, removal of the epithelium from rabbit intrapulmonary bronchi, abolished methacholine-induced relaxation of the rat aorta.

These results demonstrate that the epithelium from rabbit intrapulmonary bronchi is capable of releasing an inhibitory factor which is receptor mediated, not a product of the cyclooxygenase pathway, nor does it activate guanylate cyclase.

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DIFFERENTIAL REGULATION BY GLUCOCORTICOIDS OF INTRACELLULAR LIPOCORTIN I, II AND V IN RAT MIXED PERITONEAL LEUKOCYTES

F. Smillie, S.H. Peers, A-J. Elderfield, C. Bolton and R.J. Flower*.
Pharmacology Group, University of Bath, Claverton Down, Bath, BA2 7AY, U.K.

Lipocortins (LC's) are a family of proteins sharing common "core" sequences but with different N-terminal domains (Pepinsky et al 1988). Although evidence suggests that LC I may be regulated by glucocorticoids in leukocytes (Wallner et al 1987) little is known concerning the effect of these drugs on this and the other members of the LC family. We now report a differential action of glucocorticoids upon LC's I, II and V in rat peritoneal mixed leukocytes.

LC's were detected in cells following separation of protein extracts by SDS-PAGE by conventional Western immunoblotting techniques using highly selective polyclonal antisera. Absolute quantitation of protein mass is impossible using this technique but the optical density (OD) of the antibody-stained bands may be used as a semi-quantitative measure. Rat peritoneal leukocytes were obtained as described (Blackwell et al 1982) from adrenalectomised or normal male Wistar rats (150-200 g) treated with either vehicle, glucocorticoid, the glucocorticoid antagonist RU486 or a combination of treatments. When pellets containing known numbers ($5x10^6$) of peritoneal cells were extracted, LC's were found in three distinct locations (a) on the external surface of the plasma membrane; (b) in the intracellular compartment; (c) as integral membrane proteins. More than 95% of the protein was found in the intracellular compartment and was conveniently extracted from lysed cells in Tris buffer (pH 7.4, 50 mM) containing 10 mM EDTA. Representative data from one of these series of experiments is given below.

Cells from rats receiving vehicle only contained easily detectable amounts of LC's I, II and V. LC I and LC II disappeared after pretreatment of rats with RU486 (10 mg/kg p.o) although LC V was unchanged. Treatment of rats with dexamethasone (0.08 mg/kg s.c) or hydrocortisone (1 mg/kg s.c) for 2h increased LC I (x1.67, x2.32 respectively) and LC II (x1.98, x2.74 respectively) as gauged by changes in O.D, but LC V was unchanged. Pretreatment of rats with RU486 completely prevented the dexamethasone-induced increases. The time course of the 'induction' was examined in adrenalectomised rats. LC I was just detectable in cells taken from these rats , LC II was virtually undetectable and LC V was present in abundance. Within 30 min of receiving dexamethasone (0.08 mg/kg s.c) the mass of LC I as judged by the O.D had risen by 3.69x and had increased to 6.52x by 2 h. Similarly LC II had increased to 28.9x within 30 min of steroid and to 43.20x by 2h. In contrast, LC V levels did not alter at all during this time. Qualitatively similar effects on LC I were observed when mixed peritoneal cells were incubated with dexamethasone (10^{-6} 10^{-9}M) *in vitro* although the subcellular location of LC I seemded different with more protein being found on the external cell membrane.

We conclude that both LC's I and II, but not the closely related species LC V, are upregulated by acute glucocorticoid treatment in rat mixed peritoneal cells.

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QUANTIFICATION OF LIPOCORTIN 1 IN BRONCHOALVEOLAR LAVAGE FLUID FROM PATIENTS WITH PULMONARY SARCOIDOSIS

NJ Goulding, SF Smith*[1], JL Godolphin, TD Tetley[1], CM Roberts[1], A Guz[1] & RJ Flower. Bath Institute for Rheumatic Diseases & Dept of Pharmacology, University of Bath, UK & [1]Dept of Medicine, Charing Cross & Westminster Medical School, London UK

Lipocortin (Lc 1; 37 kDa) is a glucocorticoid-inducible anti-inflammatory protein (Flower, 1988) present in bronchoalveolar lavage fluid (BALF) from man (Smith et al, in press). Lc 1 may be made in the lung or may diffuse (or be transported) from the blood to the lung. Assay of Lc 1 levels in BALF from patients with pulmonary sarcoidosis may help to answer this question as, in these subjects, pulmonary epithelial permeability is increased, causing a selective rise in plasma proteins eg. albumin in BALF (Jacobs et al, 1985).

The study was carried out on ten patients, none taking systemic glucocorticoids. Five controls (CON; 1 current, 1 ex-smoker; 3 male; aged 25 - 60 yrs, median 39 yrs) were patients who were found to have normal lungs. The other five patients (SAR; 1 current, 1 ex-smoker; 2 male; aged 19 - 43 yrs, median 41 yrs) had pulmonary sarcoidosis. BALF was collected, cell profile determined and extracellular albumin levels assayed as previously described (Smith et al, 1985). Lc 1 was assayed by an enzyme immunoassay developed by NJG and JLG. Incubations were 100 ul/well for 1h at 37°C unless otherwise stated. Washes were performed after each step. Nunc Immunolon type I microtitre plates were coated with monoclonal antibody (2 ug/well) to human recombinant Lc 1 and incubated for 18 h at 4°C. After blocking with 1% foetal calf serum, BALF or recombinant human Lc 1 (0.125 ng - 4 ug/well) was added and incubated. Samples were next incubated with rabbit anti-human recombinant Lc 1, then with goat anti-rabbit IgG conjugated to alkaline phosphatase and finally, with 1 mg/ml disodium nitrophenylphosphate in Voller's buffer, pH 9.6 for 40 min at 18°C, and the absorbance at 405nm determined in an automated plate reader.

	BALF Vol. (ml)	Cell No. (x 10^6/BALF)	Lymphocytes (% Cells)	Albumin (ug/ml)	Lc 1 (ng/ml)	Serum cortisol (nmol/ml)
CON	133	17.5	7	18	19	215
	(63-142)	(5.4-34.3)	(3-20)	(15-138)	(11-67)	(165-338)
SAR	125	45.5~	59~	93~	22	311
	(98-140)	(27.8-57.8)	(12-77)	(64-289)	(14-33)	(127-367)

~>control, $p<0.05$, one-tailed Wilcoxon Rank Sum Test; median (range)

Although the concentration of albumin was markedly increased in BALF from patients with sarcoidosis, concentrations of Lc 1 were not. This suggests that Lc 1 is locally produced in the lung, although the exact site remains unknown. Lc 1 levels were related to serum cortisol (r_s = 0.6182, $p<0.05$). We believe this is the first accurate quantification of Lc 1 in clinical material.

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SPECIES DIFFERENCES IN THE ANTI-INFLAMMATORY POTENCY OF DEXAMETHASONE

C.J. Whelan, Department of Peripheral Pharmacology, Glaxo Group Research Ltd., Ware, Herts SG12 ODJ U.K.

Glucocorticoids are claimed to be relatively weak anti-inflammatory agents in the guinea-pig (Brattsand et al., 1985), although comparative studies with other species under the same conditions have not been carried out. In this report, the anti-inflammatory effects of dexamethasone on zymosan (Z)-induced polymorphonuclear (PMN) cell accumulation and plasma protein extravasation (PPE) in guinea-pig skin have been compared with that in the rat.

Guinea-pig and rat PMN cells were isolated from glycogen-induced peritoneal exudates by centrifugation on histopaque 1077 (Sigma) at 200g for 20 min. The cells were washed twice in heparinised (10U/ml)-Tyrode's solution and incubated with ^{111}indium oxine (5.55MBq) for 15 min. After labelling, PMN cells were washed and resuspended in heparinised-Tyrode's solution (2ml) containing iodinated albumin ($^{125}I_2$, 0.37MBq). Animals were anaesthetised with ketamine (40mg/kg i.m.) and xyalzine (8mg/kg i.m.) and each was given an intracardiac injection of 0.3ml PMN cell suspension (containing approximately $1x10^5$ cells) and a series of intradermal injections of saline or Z in saline into the abdominal skin. The animals were killed after 4 hr and the injection sites removed with an 18mm hollow punch. The ^{111}In and $^{125}I_2$ content of the biopsies and aliquots of blood and plasma were then determined. From these data, the number of PMNs in each biopsy (expressed as $x10^3$ cells/site) and PPE (expressed as µl plasma) were calculated.

Intradermal Z (0.2mg/site) induced PMN cell infiltration and PPE in guinea-pig skin. In rats, Z caused a greater number of PMN cells to accumulate. Dexamethasone, at doses up to 5mg/kg s.c. (given 3hr prior to Z) did not significantly reduce PMN cell accumulation or PPE in the guinea-pig. Higher doses of dexamethasone (50 and 150mg/kg s.c.), however, significantly ($P<0.05$) reduced cell accumulation from 12.9±4.0 (n-5) and 13.5±1.8 (n=6) $x10^3$ cells to 5.3±2.7 (n=5) and -0.24±0.8 (n=6) respectively. Similarly, these doses of dexamethasone significantly ($P<0.05$) reduced PPE from control values of 5.0±0.5 (n=5) and 5.8±1.7 (n=6) µl plasma to 1.7±0.8 (n=5) and -0.7±0.8 (n=6). Pretreatment of guinea-pigs with actinomycin D (5mg/kg s.c. 3 and 1.5hr prior to Z) attenuated the anti-inflammatory effects of dexamethasone. In rats, dexamethasone (5.0mg/kg s.c.) significantly ($P<0.05$) reduced cell accumulation from 108.0±26.8 (n=8) to 29.6±9 (n=8) $x10^3$ cells and PPE from 8.2±3.5 (n=10) to -0.33±0.6 (n=10) µl plasma, confirming the findings of Tsurufuji et al (1984).

The experiments described above demonstrate that dexamethasone is approximately 30-fold less potent as an anti-inflammatory agent in the guinea-pig than in the rat. However, the anti-inflammatory actions of the high doses of dexamethasone required in the guinea-pig are attenuated by the RNA synthesis inhibitor actinomycin D, suggesting that these actions are mediated via glucocorticoid receptors (Tsurufuji et al., 1979).

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DISRUPTION OF EQUINE BOWEL MOTILITY BY ENDOTOXIN - EVIDENCE OF A ROLE FOR PROSTAGLANDINS AND PAF

J.N. King* & E.L. Gerring (Introduced by F. Cunningham), Department of Surgery & Obstetrics, Royal Veterinary College, Hawkshead Lane, Hatfield, Herts. AL9 7TA.

Postoperative ileus (POI) is a common and serious complication of abdominal surgery in the horse. There is evidence that endotoxin may be involved since in most cases of POI ischaemic bowel is or has been present (Hunt, et al. 1986). The effects of endotoxin and its possible mode of action have therefore been investigated in an equine model of ileus.

A dose of endotoxin (0.1μg/kg i.v.) equivalent to that detected in clinical cases (King & Gerring, 1988) was used to produce marked inhibition of gastric and colonic motility as well as abnormal motility patterns in the small intestine of ponies implanted with gastrointestinal electrodes and strain gauges. These responses were antagonised by pretreatment with the cyclo-oxygenase inhibitors flunixin (1.1 mg/kg i.v.) and phenylbutazone (4.4 mg/kg i.v.) and with a selective PAF receptor antagonist WEB-2086 (6.6 mg/kg i.v.). Motility patterns similar to those observed with endotoxin were induced by the intravenous infusion of PG-E2 (3.3 & 10.0 μg/kg) and less markedly with PG-12 (2 & 5μg/kg) but not with PG-F2$_{\alpha}$(3.3 & 10.0μg/kg).

Table 1. Values expressed as a % of the control contraction product (amplitude x rate of contractions in arbitary units). Mean (s.e. mean)

Control	Endotoxin (End)	PBZ + End	Flunixin + End	WEB-2086 + End	PG-E2	PG-12	PG-F2$_{\alpha}$
n = 6	n = 6	n = 3	n = 3	n = 3	n = 3	n = 3	n = 3
Stomach							
100.0	18.47##	93.6**	66.0*	79.4**	43.5##	84.0	128.6
(5.9)	(3.0)	(11.2)	(12.1)	(5.0)	(4.5)	(5.4)	(8.1)
Ascending colon							
100.0	42.0##	133.1**	73.7	93.3	30.7##	71.6	155.4
(16.1)	(13.2)	(14.9)	(9.6)	(17.8)	(5.8)	(7.9)	(14.8)

##p<0.001 vs control, *p<0.01 & ** p<0.001 vs endotoxin.

These results suggest that the action of endotoxin on equine gut motility may be mediated, at least in part, by prostaglandins and PAF.

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EFFECT OF DEXAMETHASONE AND KETOPROFEN ON THE PYROGENIC RESPONSES TO HUMAN RECOMBINANT INTERLEUKIN-1α(IL-1α) AND INTERLEUKIN 1β(IL-1β)

J. Davidson, A.S. Milton, & D. Rotondo, Department of Pharmacology, University of Aberdeen, ABERDEEN AB9 1AS Scotland, U.K.

Interleukin-1 is thought to be an endogenous mediator of fever in response to infectious agents and other exogenous pyrogens such as the purified cell-wall components of gram -ve bacteria, lipopolysaccharide, and the synthetic double-stranded ribonucleic acid, polyinosinic:polycytidylic acid. Recently molecular cloning techniques have revealed that there are two distinct genes in the human genome which encode for two forms of the IL-1 molecule, a form with an isoelectric point (pI) of 5 termed IL-1α and a pI 7 form termed IL-1β, (Auron *et al*, 1984). The active forms of IL-1α and IL-1β both have a molecular weight of 17,000 and appear to bind to the same receptor with equal affinities, (Kilian *et al*, 1986) and also have similar biological activity even although their amino acid homology is relatively low being only 26%. In the present study we have investigated the pyrogenicity of both recombinant human IL-1α and IL-1β. Recently we have shown that the synthetic glucocorticoid dexamethasone and the non-steroidal anti-inflammatory agent ketoprofen are antipyretic towards lipopolysaccharide, polyinosinic: polycytidylic acid and endogenous pyrogen/IL-1, (Abul *et al*, 1987; Rotondo *et al*, 1988). We have therefore studied the effects of dexamethasone and ketoprofen on the pyrogenic responses to IL-1α and IL-1β.

Male Dutch rabbits (2.0-2.3 kg) were lightly restrained in stocks and their rectal temperatures constantly monitored using thermistor probes. Solutions of IL-1 were prepared (under aseptic conditions) in sterile saline. Doses of IL-1 were measured in International Reference Units (U) as described by the National Institute for Biological Standards (from where the IL-1α and IL-1β were obtained) each U being equivalent to 10 pg of either IL-1α or IL-1β. Dexamethasone and ketoprofen were dissolved in sterile saline and filtered through 0.22 μm filters prior to use. All solutions were prepared immediately prior to use and were administered via the marginal ear vein.

The threshold dose of IL-1α and IL-1β required to produce a reproducible pyrogenic response was 500 U/kg and 100 U/kg respectively. This produced a maximal rise in rectal temperature (ΔT_{max}) of 0.21 ± 0.02 °C and 0.21 ± 0.05 °C for IL-1α and IL-1β respectively, (means ± SEM for n = 4). The ΔT_{max} for all doses used always occurred 45 minutes after administration of IL-1. Maximal thermal response indices over 2 hours (TRI_2) were observed with doses of 5,000 U/kg for both IL-1α and IL-1β which produced TRI_2 values of 0.916 ± 0.085 and 1.220 ± 0.131 and ΔT_{max} values of 0.67 ± 0.06 °C and 0.97 ± 0.12 respectively, (means ± SEM for n = 4). The TRI_2 for saline controls was 0.257 ± 0.051. Ketoprofen (3 mg/kg) given s.c. 15 minutes beforehand completely abolished the pyrogenic response to either 2,500 U/kg of IL-1α or 500 U/kg of IL-1β which produced ΔT_{max} of 0.61 ± 0.03 and 0.60 ± 0.06 and TRI_2 values of 0.734 ± 0.071 and 0.698 ± 0.047 respectively (means ± SEM for n = 4). Dexamethasone (3 mg/kg) given i.v. 1 hour prior to either 2,500 U/kg of IL-1α or 500 U/kg of IL-1β reduced the TRI_2 values to 0.425 ± 0.098 and 0.45 ± 0.042 respectively (both $P<0.02$) and the ΔT_{max} to 0.37 ± 0.08 and 0.31 ± 0.12 (both $P<0.05$) respectively, (means ± SEM for n = 4).

These data show that IL-1β has a greater pyrogenic action than IL-1α which is in the order of 1.5-fold based on the ΔT_{max} value for 2,500 U/kg of either IL-1. It is not certain why IL-1α and IL-1β have different pyrogenic potencies if they have equal affinities for most IL-1 receptors. The ketoprofen and dexamethasone data indicate that prostanoids are involved in the pyrogenic responses and it is possible that the different pyrogenic potencies may be related to their relative ability to stimulate prostanoid biosynthesis.

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PHOTODYNAMIC DRUG ACTION ON RAT PANCREATIC ACINI

E.K. Matthews* & Z.J. Cui, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD

Halogenated fluorescein molecules, when photon-activated, permeabilize the cell membrane via the generation of singlet oxygen and elicit a sustained calcium-dependent contraction of smooth muscle (Matthews & Mesler, 1984). We have now investigated the halogenated fluorescein derivative, rose bengal (RB) and a new photodynamic agent, sulphonated aluminium phthalocyanine (SALPC) for their ability to permeabilize pancreatic acinar cells both to establish their precise mechanism of action on secretory cells and because SALPC has potential use as an antitumour agent.

Dispersed acini were isolated from the rat pancreas by collagenase digestion, mixed with a suspension of Biogel (P2) beads, loaded into a small column, perifused with a HEPES buffered medium at 37°C and illuminated when required with a quartz-halogen light source (visible spectrum >400nm) or He-Ne laser (8mW; 633nm). Stimulation of secretion was assessed by measuring amylase released from the acini as a percentage of total cellular amylase. Membrane permeabilization kinetics were determined by the leakage of cytoplasmic lactate dehydrogenase (LDH) and by the efflux of ^{86}Rb from preloaded acini.

Light alone ($\lesssim 1.84 \times 10^4$ lux), or SALPC ($\lesssim 1\mu M$) in the absence of light, had no effect on the pancreatic acini but SALPC, when illuminated, caused a dose-dependent (10nM to 1μM) and light intensity-dependent (1×10^2 to 1.84×10^4 lux) release of amylase. Illumination at a constant 4.5×10^3 lux for 10 min caused a rapid five- to six-fold increase in pancreatic amylase release ($n \gtrsim 3$) but the efflux of ^{86}Rb and loss of cytosolic LDH were delayed in onset. Similar results were obtained when coherent monochromatic laser light (633nm) were used. In contrast, the cholinergic agonist bethanechol, $10^{-4}M$, evoked a rapid five-fold increase in amylase release and a small efflux of ^{86}Rb, but is known not to cause the loss of cytosolic LDH.

Separate experiments demonstrated that singlet oxygen is produced in high quantum yield in aqueous solution by the photon activation of SALPC or RB. Furthermore, ionized Ca^{2+} (10μM) was found to enhance the photodynamic release of amylase by RB, 1μM, from 1.8±0.3 to 3.1±0.2 of total amylase ($P<0.02$).

Ultracytological evidence from electron microscopy confirmed that the pancreatic acinar cells maintained their structural integrity after the photodynamic action of SALPC.

In conclusion, these results are consistent with the photodynamic generation by SALPC or RB of highly reactive singlet oxygen which causes photo-oxidation of membrane components, an increased permeability of the acinar cell membrane and a Ca^{2+}-dependent secretion of amylase.

Z.J. Cui is a British Council Scholar.

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HIGH DOSE PURIFIED EICOSAPENTAENOIC ACID (EPA): EFFECTS ON LEUKOTRIENE SYNTHESIS AND PLASMA LIPIDS

A.B.Hawthorne*, B.Filopowicz, T.Edwards, T.K.Daneshmend, & C.J.Hawkey, Dept of Therapeutics, University Hospital, Nottingham, NG7 2UH.

Fish oils have antithrombotic, anti-inflammatory and lipid-lowering effects. Most clinical studies have used a mixture of triglycerides of EPA and docosahexaenoic acid (DHA), the EPA component being more potent than DHA (Terano et al, 1987). Highly purified EPA is obtained from fish oil by fractionation of the ethyl ester. A larger dose of EPA may then be given in a smaller volume. The optimal dose required is not known, and so we have studied the biochemical effects and tolerance of 6g and 18g of EPA ethyl ester daily.

93% pure EPA ethyl ester was given to 6 healthy subjects as a diet supplement in a dose of 6g daily (7.2ml) for 6 weeks, a 4 week break, and then 18g daily (21.5ml) for a further 6 weeks. Blood was taken after overnight fast, for plasma lipids, neutrophil studies and red cell membrane fatty acids. Following dextran sedimentation of red cells, neutrophils were isolated, and leukotriene(LT) production stimulated by ionophore A23187 at 37°C for 5 min. Supernatants were analysed by reverse phase HPLC (Wilkinson et al, 1985). Red cell lipids were extracted, methylated and analysed by gas chromatography.

Results are expressed as mean (s.e.mean), (*p<0.05, **p<0.01)

Weeks	0	(6g EPA) 6	10	(18g EPA) 16
Red cell membrane fatty acids (% total lipids)				
EPA	1.1(0.3)	3.2(0.3)**	1.5(0.2)	5.7(0.6)**
DHA	5.3(0.2)	4.0(0.2)**	4.6(1.0)	3.5(0.4)**
Leukotrienes ($ng/10^7$ cells)				
LTB4	97.0(8.7)	52.0(15)*	88.0(12)	38.0(10)*
LTB5	<8.0	9.3(1.0)	<8.0	17.3(3.9)
5-HETE	99.0(15)	45.0(12)	75.0(31)	41.0(6.0)
5-HEPE	<7.5	16.0(4.5)	8.8 (1.1)	38.0(5.6)*
Plasma lipids (mM)				
Total chol.	5.2(0.3)	4.9(0.3)	4.9(0.3)	4.5(0.3)*
VLDL chol.	0.38(0.1)	0.29(0.08)	0.35(0.11)	0.27(0.09)
LDL chol.	3.9(0.3)	3.6(0.4)	3.5(0.2)	3.4(0.4)**
Total trig.	1.1(0.2)	0.75(0.16)*	0.91(0.2)	0.83(0.2)**

(chol.= cholesterol, trig.= triglyceride, (V)LDL= (very)low density lipoprotein, 5-HET(P)E= 5-hydroxyeicosatetra(penta)enoic acid)

High dose EPA gave greater membrane incorporation, more inhibition of leukotriene synthesis and reduction in plasma lipids, despite the very poor absorption of the high dose, manifested by the passage of fat in the stool, (2 subjects were unable to tolerate the 18g dose for this reason). High dose of EPA ethylester is limited by absorption.

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Ca^{2+} ANTAGONISTS AND HYDRALAZINE ALTER 5-HT MEDIATED BEHAVIOUR IN RODENTS

A. Richard Green*, Rosemarie J. De Souza, Elaine M. Davies & Alan J. Cross, Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ.

Recently Boullin & Grahame-Smith (1987) reported that administration of various Ca^{2+} antagonists to rats pretreated with the monoamine oxidase inhibitor tranylcypromine resulted in the animals displaying the complete 5-HT behavioural syndrome. The Ca^{2+} antagonists also enhanced the syndrome when it had been induced by injection of tranylcypromine and L-tryptophan. No enhancement of agonist induced behaviour was observed. We have now investigated further the interaction of Ca^{2+} antagonists with 5-HT mediated behaviour.

Administration of felodipine (10 and 35mgkg^{-1} i.p.) to tranylcypromine (10mgkg^{-1} i.p.) pretreated rats resulted in the appearance of the 5-HT behavioural syndrome (head weaving, forepaw treading, flat body posture) 90 min later. Felodipine (10mgkg^{-1}) pretreatment also enhanced the tranylcypromine/L-tryptophan behavioural syndrome, an effect not antagonised by administration of Bay K8644 (1mgkg^{-1} i.p.). The behavioural syndrome evoked by 8-OH-DPAT (0.75mgkg^{-1} s.c.), the 5-HT agonist, was unaltered by felodipine pretreatment.

No evidence was obtained for felodipine (35mgkg^{-1}) altering the rate of 5-HT synthesis in vivo or for felodipine (10μM) altering the K^+ evoked release of endogenous 5-HT from slices prepared from either the frontal cortex or brain stem.

Head twitch behaviour induced in mice by injection of 5-Methoxy-N, N-dimethyltryptamine (5-MeODMT; 5mgkg^{-1}) (see Green & Heal, 1985) was markedly inhibited by several Ca^{2+} antagonists given 15 min earlier (Table). This effect was not reversed by injection of Bay K8644 (Table). While both verapamil (IC_{50} : 3μM) and nicardipine (IC_{50} : 8μM) were reasonably potent inhibitors of [^{3}H]-ketanserin binding to membrane prepared from mouse frontal cortex, felodipine and nifedipine only inhibited 5-HT_2 receptor binding in the millimolar range.

Table Effect of Ca^{2+} channel antagonists and hydralazine on 5-MeODMT induced head twitch behaviour.

Treatment	Dose (mgkg^{-1})	% Inhibition Head Twitch
Saline/DMSO	—	0 ± 18
Nifedipine	20	83 ± 19*
Nicardipine	25	74 ± 21*
Felodipine	4	61 ± 12*
Verapamil	25	77 ± 13*
Bay K8644	1	8 ± 35
Felodipine + Bay K8644	4	80 ± 11†
Verapamil + Bay K8644	25	75 ± 7†
Hydralazine	5	62 ± 26*

*Different from control $p < 0.05$ or better
†Not different from Ca^{2+} antagonist in absence of Bay K8644

Interestingly hydralazine (5mgkg^{-1} i.p.) pretreatment also produced the behavioural syndrome in tranylcypromine pretreated rats, enhanced the tranylcypromine/L-tryptophan syndrome and inhibited 5-HT_2 head twitch behaviour in mice, without being a 5-HT_2 receptor antagonist.

These data indicate that Ca^{2+} antagonists and hydralazine can have marked effects on central 5-HT function. The mechanisms involved however are at present obscure.

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EVIDENCE FOR 5-HT_{1C} RECEPTOR MEDIATION OF ANOREXIA IN THE RAT

J.C. Neill* & S.J. Cooper, School of Psychology, University of Birmingham, Birmingham B15 2TT.

The effect of (+)-fenfluramine to reduce palatable food consumption in non-deprived rats is probably mediated by selective activation of central 5-HT_1-like receptors (Neill & Cooper, 1989). In freely-feeding rats, stimulation of post-synaptic 5-HT_{1B} and 5-HT_{1C} receptors leads to decreases in food intake (Kennett & Curzon, 1988; Kennett et al., 1987). The present study was designed to investigate anorectic and possible hyperphagic effects of selective 5-HT agonists in the palatable food intake model. The putative 5-HT_{1B} agonist RU 24969, the 5-HT_{1B}/5-HT_{1C} agonists m-chlorphenylpiperazine (mCPP) and trifluoromethylphenyl-piperazine (TFMPP), the selective 5-HT_{1B} agonist CGS 12066B (Neale et al., 1987), and MK 212, a selective 5-HT_{1C} receptor agonist (Conn & Sanders-Bush, 1987) were tested.

Adult, male hooded rats were adapted to eating a palatable mash (50 ml sweetened condensed milk: 150 ml ground rat maintenance diet: 200 ml distilled water) in daily 30 min sessions. Food and water were available ad lib at all times. Animals were tested under two conditions: a standard procedure (high baseline) to test for anorexia, and a partial pre-satiation procedure (low baseline) to test for hyperphagia (Neill & Cooper, 1988). The drugs were administered i.p., 30 min prior to the feeding test sessions. At least 6 rats were tested at each dose. Data were analysed by ANOVA for independent groups, followed by Dunnett's t-test.

Drug	Standard: dose-range ($mg.kg^{-1}$)	M.E.D. ($mg.kg^{-1}$)	Pre-satiated: dose-range ($mg.kg^{-1}$)	M.E.D. ($mg.kg^{-1}$)
MCPP	0.1 - 10.0	1.0	0.03 - 3.0	1.0
TFMPP	0.3 - 10.0	1.0	0.03 - 3.0	1.0
MK 212	0.3 - 3.0	1.0	0.1 - 3.0	3.0
RU 24969	0.1 - 1.0	1.0	0.01 - 0.3	n.e.
CGS 12066B	0.03- 3.0	n.e.	0.03 - 3.0	n.e.

M.E.D. = min. effective dose to reduce food intake ($p<0.05$). n.e. = no effect.

mCPP, TFMPP and MK 212 produced significant dose-dependent reductions in palatable food consumption under both procedures. CGS 12066B had no effect, either to increase or decrease food intake. RU 24969 reduced intake at 1.0 $mg.kg^{-1}$, but this coincided with induction of behavioural stereotypy. The results indicate that selective activation of the 5-HT_{1C} receptor subtype may be necessary to obtain an anorectic effect in rats consuming a sweetened, palatable diet. In contrast to effects of putative 5-HT_{1A} agonists (Neill & Cooper, 1988), there was no evidence of hyperphagic drug effects in partially pre-satiated rats.

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5-HT_{1C} RECEPTORS MAY MEDIATE THE ANXIOGENIC-LIKE ACTION OF MCPP IN THE RAT SOCIAL INTERACTION TIEST

Kennett*, G.A., Whitton+, P., Shah+ K. and Curzon+, G. Beecham Pharmaceuticals, Harlow, Essex, CM19 5AD and +Institute of Neurology, Queen Square, London, WC1N

1-(3-chlorophenyl)piperazine (mCPP) and 1-[3-(trifluoromethyl)phenyl]piperazine (TFMPP) have behavioural effects mediated by $5HT_{1C}$ receptors (Kennett and Curzon, 1988a,b) for which both have now been shown to have some selectivity (Hoyer, 1988). As mCPP induces anxiety in humans (Kahn et al, 1988) we have studied whether it has anxiogenic effects in rats and whether these are $5HT_{1C}$ receptor mediated.

Singly housed male Sprague Dawley rats (200-250g) were placed in a social interaction box (60 x 27 x 30cm) for 10 min (daily x 2). On day 3, rats were injected s.c. in weight matched pairs with vehicle [saline (-propranolol, CDP), or 0.0175M acetic acid in saline neutralised to pH 6.5 with 5N NaOH] or antagonist followed 20 min later by mCPP or saline i.p. After a further 20 min they were placed together with their weight matched pair mate in the interaction box for 15 min under red light and videotaped.

TABLE 1: Effect of pretreatment with chlordiazepoxide (CDP) (5mg/kg i.p. daily x 5) or mianserin (2mg/kg s.c.) on mCPP (0.5mg/kg i.p.) induced deficits in social interaction. All points means ± SEM (n=6-8 per group)

Pretreatment	Treatment	Total interaction time (S)	Locomotion (squares crossed)
Chronic vehicle	saline	293 ± 36	122 ± 10
Chronic vehicle	mCPP	123 ± 21**	105 ± 15
Chronic CDP	saline	225 ± 18	129 ± 8
Chronic CDP	mCPP	275 ± 18++	120 ± 9
Vehicle	saline	257 ± 36	96 ± 10
Vehicle	mCPP	113 ± 19**	103 ± 14
Mianserin	saline	219 ± 21	143 ± 8*
Mianserin	mCPP	220 ± 20+	120 ± 9

Significantly different from vehicle + saline *P<0.05, **P<0.01, or from vehicle + mCPP treated group, ++P<0.01 by Newman Keuls test following significant 1 way ANOVA.

mCPP and TFMPP (0.1-1.0 mg/kg) had anxiogenic-like effects reducing total interaction by up to 70% (P<0.01) and 55% (P<0.01) respectively, but not locomotion (except mCPP at 1mg/kg). This effect of mCPP was blocked by chronic chlordiazepoxide pretreatment (5mg/kg daily x 5 last dose 20 min prior to mCPP; Table 1). It was also blocked by the non-specific 5HT antagonists, mianserin (2mg/kg; Table 1), metergoline (2.5mg/kg) and cyproheptadine (2mg/kg), which all have high affinity for $5HT_{1C}$ receptors (Hoyer, 1988). However it was not blocked by the $5HT_2$ antagonists ketanserin (0.2mg/kg) and ritanserin (0.6mg/kg), nor by the $5HT_{1A}$ and $5HT_{1B}$ antagonists cyanopindolol (6mg/kg) and (-) propranolol (16mg/kg) despite the latters reported high affinity for $5HT_{1C}$ sites (Hoyer, 1988). It was also not blocked by the $5HT_3$ antagonist ICS 205-930 at high (1mg/kg) dose. In conclusion mCPP and TFMPP have anxiogenic-like effects in the rat social interaction test which in the case of mCPP at least may be mediated by $5HT_{1C}$ receptors.

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CHRONIC ETHANOL INCREASES EXCITABILITY OF HIPPOCAMPAL CELL BODIES; EFFECTS DECREASED BY CHRONIC NITRENDIPINE TREATMENT

M. A. Whittington* & H. J. Little, Department of Pharmacology, The Medical School, University Walk, Bristol, BS8 1TD.

Long term intake of ethanol is known to cause a withdrawal syndrome on cessation of treatment. Dihydropyridine calcium channel blockers given concurrently, during ethanol chronic treatment, or as a single dose on removal of ethanol, prevented the withdrawal-induced convulsive behaviour (Whittington & Little 1988). These treatments also decreased the hyperexcitability seen on withdrawal from ethanol when recordings were made from hippocampal slices *in vitro*, when orthodromic stimulation was used (Whittington & Little, 1989). In order to clarify the mechanism of action of the dihydropyridines on withdrawal, we have now measured field potentials after antidromic stimulation in hippocampal slices removed from mice immediately after chronic ethanol treatment, and the effects of the dihydropyridine calcium channel antagonist, nitrendipine.

Male mice, C57 strain, 27-32g, were given ethanol, 24% v/v, as sole fluid, for twelve weeks. Nitrendipine was given to some of the mice as a 1.77mM solution in the ethanol drinking fluid (intakes: ethanol 11 ± 0.2 g/kg/day, nitrendipine 56 ± 1 mg/kg/day, mean ± s.e.m.), or to the slices as a 1μM solution in the bathing medium. Controls drank water. Hippocampal slices were taken between 9 and 10am, without prior withdrawal from ethanol, and perfused with standard Ringer solution (containing 2mM calcium). Extracellular recording from the CA1 area, using antidromic stimulation of the alveus, was started after the slices had settled in the recording chambers for 30 min. Thresholds for production of first and multiple population spikes were measured every hour for 7h. Paired pulse inhibition was measured with a range of pulse intervals between 5 and 30ms, with stimulus levels from threshold to maximum population spike. The areas of population spikes were measured by computer analysis. Results, expressed as mean ± s.e.m., from slices having different treatments were compared by analysis of variance (n = 4 - 5 each group).

Significant decreases in population spike and multiple spike thresholds were seen in the field potentials of slices from mice treated with chronic ethanol, compared with controls, in the 3 - 7h timeperiod. Concurrently administered nitrendipine (Eth + Chr.nitr.) significantly reduced the ethanol-induced changes in thresholds. A significant decrease in paired pulse inhibition was seen in slices from ethanol treated mice when the pulse interval was less than 10ms; no change was seen with larger interpulse intervals. Slices from animals given both ethanol and nitrendipine chronically also showed a significant reduction in this withdrawal-induced decrease in inhibition.

(h=from tissue removal)	Controls	Ethanol	Eth + Chr. nitr.	Eth + Perf. nitr.
Single spike threshol, μA (5h)	19 ± 1	14 ± 1	19 ± 3	17 ± 1
Multiple spike threshold, μA (5h)	128 ± 11	78 ± 6	97 ± 4	91 ± 4
Paired pulse inhibition (3h)	33 ± 6	59 ± 5	40 ± 5	55 ± 2
(% second/first spike at 5ms separation)				
P values compared with ethanol group:		Controls	Eth + Chr. nitr.	Eth + Perf. nitr.
Single spike threshold (3 - 7h)		0.02	0.05	0.7
Multiple spike threshold (0 - 7h)		0.004	0.02	0.05
Paired pulse inhibition (1 - 3h)		0.001	0.001	0.2

These results show that withdrawal from ethanol decreased the firing thresholds of CA1 pyramidal cells when antidromic stimulation was used. This indicates that changes in the pyramidal cell body region may be responsible for the withdrawal hyperexcitability. Paired pulse data showed a decrease in the initial component of recurrent inhibition on withdrawal from ethanol, suggesting a decrease in $GABA_A$ mediated responses. Both these types of changes were decreased by chronic nitrendipine, providing further evidence of a role for dihydropyridine-sensitive calcium channels in withdrawal hyperexcitability.

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DEFEAT ANALGESIA IN MICE IS DIFFERENTIALLY AFFECTED BY ACUTE AND CHRONIC TREATMENT WITH THE ANTI-PANIC AGENT, ALPRAZOLAM

J.K. Shepherd* and R.J. Rodgers, Pharmacoethology Laboratory, Department of Psychology, University of Bradford, West Yorkshire BD7 1DP.

In the present study, the effects of acute and chronic alprazolam (0.05-2mg/kg), an anti-panic agent, on basal nociception and defeat analgesia in male mice have been assessed. The chronic regimen was included to control for possible sedative effects and to provide a closer clinical parallel (Johnston & File 1988).

8-12 week old male DBA/2 mice (Bantin & Kingman, Hull), housed 10/cage in a temperature controlled room (24±1°C), were used. All testing was conducted under dim red light during the dark phase of a 12h reversed LD cycle. Pain latencies, taken both before and after defeat, were determined by traditional tail-flick assay using a cut-off of 8s. For defeat experience, intruder DBA/2 mice were individually placed into the home cage of an aggressive resident conspecific and removed immediately upon the display of a species-typical defeat posture or, in the absence of defeat, after a maximum encounter duration of 270s. Alprazolam was ultrasonically dispersed in a physiological saline/Tween 80 vehicle and administered in a volume of 10ml/kg(i.p.) thirty min before testing. For the chronic study, alprazolam was given daily for 9 days prior to an identical test procedure. Data were analyzed by ANOVA and appropriate follow-up tests, and are given as mean tail-flick latencies.

ACUTE TREATMENT	0	0.05	0.1	0.5	mg/kg alprazolam
Pre-defeat	2.7±0.2	3.1±0.2	3.3±0.2	2.9±0.2	
Post-defeat	4.1±0.3***	3.9±0.3*	4.3±0.6**	4.2±0.6***	

CHRONIC TREATMENT	0	0.1	0.5	1.0	2.0 mg/kg alprazolam
Pre-defeat	2.0±0.1	2.2±0.3	1.8±0.1	1.8±0.1	2.0±0.1
Post-defeat	3.7±0.5***	3.9±0.5***	2.2±0.1	2.1±0.1	2.4±0.3

* $p < 0.02$ ** $p < 0.01$ *** $p < 0.001$ versus pre-defeat

Statistical analysis showed that, over the dose range used, alprazolam was without significant effect on the tail-flick assay following either acute or chronic treatment (Acute: $F(5,48)=0.84$, NS, Chronic: $F(4,37)=0.15$, NS). In the defeat model, and possibly due to sedation, the display of defeat was totally disrupted by acute treatment with 1-2mg/kg alprazolam. As shown in the above table, the potent analgesia observed in the vehicle group was unaffected by lower doses (0.05-0.5mg/kg). Although, in the chronic study, activity measures confirmed the development of tolerance to the sedative effects of high doses of the compound, a marked dose-dependent failure to display defeat was maintained. Despite this finding, analysis of tail-flick data showed that analgesia was significantly blocked by 0.5-2.0mg/kg. While further studies are required, present findings suggest that the resident-intruder paradigm may prove useful as an animal model of panic disorder.

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ANALGETIC EFFECTS OF ELEVATED PLUS-MAZE EXPOSURE IN MICE: INFLUENCE OF NALTREXONE AND MORPHINE TOLERANCE

C. Lee* and R.J. Rodgers, Pharmacoethology Lab., Department of Psychology, University of Bradford, West Yorkshire BD7 1DP.

Anxiety may be an important factor in several non-opioid forms of environmental analgesia in rodents, an hypothesis supported by our recent observation of pain inhibition in male mice following exposure to the elevated plus-maze test of anxiety (EPM). The present studies assessed the influence of opiate receptor manipulations on EPM analgesia.

16-19 week old male DBA/2 mice (Bantin & Kingman, Hull) were housed 10/cage in a temperature-controlled room (24±1°C) under a 12h revered LD cycle. Testing was conducted under dim red light during the dark phase of the cycle. The EPM was based on that validated for mice by Lister (1987). Nociceptive latencies were assessed by tail-flick assay (cut-off=8s) both before and after EPM exposure. Naltrexone hydrochloride (NTX; Endo Labs. Inc.) and morphine sulphate were dissolved in 0.9% saline which, alone, served as vehicle control. Injections were given i.p. in a volume of 10ml/kg. For both studies, a 5-min EPM exposure period was used, with animals initially placed on the central square of the apparatus. In Expt. 1, mice were treated with NTX (0, 0.1, 1.0 or 10.0mg/kg) 20 min prior to testing. In Expt. 2, untreated mice were exposed to the EPM on Day 1, following which they received 7 daily injections of saline or 7.5mg/kg morphine. Animals were then challenged with EPM exposure on Day 9. Data were analyzed by ANOVA and appropriate follow-up tests.

Table I shows that EPM exposure resulted in an enduring (≥40min) analgesia that was not blocked by NTX. Indeed, the highest dose of NTX produced a significant increase in pain inhibition. This effect may be attributed to an anxiogenic effect of NTX, as revealed by behavioural analysis (reduced open arm activity, $p<0.025$).

TABLE I Effect of naltrexone on EPM analgesia. Data are mean tail-flick latencies.

Condition	Pre-EPM	0	5	10	20	40 post
Saline	2.7±0.3	2.3±0.2	3.6±0.4*	3.8±0.4*	4.1±0.3*	3.6±0.3*
0.1mg/kg NTX	2.8±0.3	2.7±0.4	4.6±0.7*	4.1±0.4*	3.8±0.4*	3.6±0.3*
1.0mg/kg NTX	3.3±0.2	3.2±0.2	4.0±0.2*	3.9±0.3	3.6±0.3	4.3±0.5*
10.0mg/kg NTX	2.8±0.3	3.0±0.2	4.9±0.5*§	5.3±0.5*§	5.1±0.7*§	4.2±0.7*

§ $p< 0.05$ versus vehicle * $p< 0.01$ versus pre-EPM

Although, in Expt. 2, EPM exposure again resulted in analgesia, this effect was not altered by chronic morphine pretreatment (see Table II) nor did this experience influence EPM behaviours.

TABLE II Effect of chronic morphine treatment on the analgetic effects of EPM exposure. Data are mean tail-flick latencies (±SEM).

Drug history	Pre-EPM	0	5	10 min post	
Saline	1.4±0.1	1.6±0.1*	1.6±0.1*	1.8±0.2**	DAY 1
Morphine	1.4±0.1	1.9±0.2**	1.9±0.2**	2.1±0.2**	
Saline	1.8±0.2	2.4±0.2**	2.0±0.2*	2.1±0.1*	DAY 9
Morphine	1.7±0.2	2.0±0.2*	2.3±0.2**	2.2±0.2**	

* $p <0.05$ ** $p <0.01$ versus pre-EPM

Present data support the view that non-opioid analgesia is a correlate of anxiety and, as such, it will be of interest to determine effects of a range of anxiolytics.

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THE EFFECT OF ATP ON CYTOSOLIC CALCIUM IN HUMAN NEUTROPHILS

Janet E. Merritt, Department of Cellular Pharmacology, Smith, Kline & French Research Ltd., The Frythe, Welwyn, Herts AL6 9AR

Addition of ATP to suspensions of human neutrophils causes a rise in cytosolic free calcium concentration ($[Ca^{2+}]_i$), as detected by changes in fluorescence of the Ca^{2+}-sensitive indicator dye fura-2. The response in the absence of extracellular Ca^{2+} was transient; $[Ca^{2+}]_i$ increased rapidly from a basal level of 100nM to a peak in excess of 1μM, and then returned to baseline. In the presence of extracellular Ca^{2+}, the rise in $[Ca^{2+}]_i$ was more prolonged presumably due to Ca^{2+} influx in addition to release from intracellular stores. Similar effects of f-met-leu-phe, PAF and leukotriene B_4 on fura-2-loaded neutrophils have already been shown, and extracellular Mn^{2+} has been used to demonstrate that these agonists stimulate a divalent cation influx (Merritt et al, 1988; 1989). ATP also stimulated Mn^{2+} entry into fura-2-loaded neutrophils, providing independent evidence for stimulated ion influx.

Of the purinergic ligands tested for stimulation of rises in $[Ca^{2+}]_i$, ATP was the most potent with an EC_{50} of 0.5-1.0μM, ADP was less effective (EC_{50} around 30μM), while 2MeSATP (a potent agonist at P_{2Y} receptors) and ADPβS were ineffective at 50μM.

A new Ca^{2+} indicator dye, fluo-3 (Minta et al, 1987), has a longer excitation wavelength which allows measurement of $[Ca^{2+}]_i$ in cells suspended in plasma; an important consideration when studying blood cells. Stimulation of fluo-3-loaded neutrophils with either ATP or f-met-leu-phe caused elevations in $[Ca^{2+}]_i$ in the presence of buffer (the method always used previously) or plasma.

This is the first demonstration of purinergic-stimulation of rises in $[Ca^{2+}]_i$ in neutrophils, suspended in either buffer or plasma. This could be an important control mechanism since there are likely to be high concentrations of ATP and ADP at sites of injury.

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THE DISCOVERY OF A TISSUE KALLIKREIN IN HUMAN NEUTROPHILS

C.D. Figueroa and K.D. Bhoola*, Department of Pharmacology, Medical School, University of Bristol, Bristol BS8 1TD.

Chemotactic factors are formed in the early stages of inflammation. On receiving chemotactic signals, polymorphonuclear (PMN) leucocytes migrate to, and accumulate at sites of inflammation. Degranulation of PMN leucocytes or secretion of the neutrophil enzymes provides an alternative mediator pathway that could control the permeability of endothelial cells to plasma proteins (Wedmore & Williams, 1981). In this research report, we describe the discovery of a tissue kallikrein in PMN leucocytes that may be released into an inflammatory milieu.

We have identified a tissue kallikrein in polymorphonuclear leucocytes (neutrophils) by immunological techniques and by kininogenase assays. Antibodies to human tissue kallikrein reacted specifically with the enzyme only in neutrophils but not in monocytes, lymphocytes, eosinophils or erythrocytes. The antibody is specific to tissue kallikrein and does not react with either plasma kallikrein or neutrophil elastase. Tissue kallikrein was localised in granules (0.15 to 0.3 μm in diameter) by the electron microscopy, immunogold technique. The immunostaining for tissue kallikrein was visualised only in the precursor neutrophils of normal bone marrow, and in the rapidly dividing myelocytes of chronic myeloid leukaemia.

Extracts of granulocytes gave a radioimmunoassay value for tissue kallikrein of 1.6 ± 0.37 ng/mg protein and 1.02 ± 0.24 ng/mg protein for cells obtained from normal and myeloid leukaemic blood, respectively. Enzymic activity was determined on the tissue kallikrein selective substrate H-D-Val-Leu-Arg-PNA; this SBTI-EDTA resistant amidase activity in extracts prepared from normal granulocytes (0.82 ± 0.08 mU/mg protein) was inhibited by antibodies to human tissue kallikrein and by trasylol. The leucocyte tissue kallikrein when incubated with human kininogen in the presence of SBTI and kininase inhibitors resulted in the formation of a substance that resembled kallidin in contracting the guinea-pig ileum, relaxing the rat duodenum and lowering the arterial blood pressure of the guinea pig. Previously, PMN leucocytes have been shown to contain two different kininogenases. One is a SBTI sensitive neutral protease (Movat et al., 1976) and the other one is a leukokinin forming acid protease similar to the cathepsins (Greenbaum, 1979). The neutrophil tissue kallikrein characterised in this study is a distinct enzyme with specific properties.

Leucocyte infiltration and cellular oedema are consistent local findings during the episodic, acute phases of inflammation in disorders like rheumatoid arthritis (Selwyn et al., 1989) and asthma (Bhoola et al., 1989). The possible release of tissue kallikrein through neutrophil degranulation and the consequential release of kallidin in such a milieu provides a further therapeutic challenge for the control of mediators that promote inflammation.

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ACUTE INFLAMMATORY EFFECTS OF A MONOCYTE-DERIVED NEUTROPHIL-ACTIVATING PEPTIDE IN RABBIT SKIN

S.J.Foster (1)*, D.M.Aked (1), J-M.Schroder (2) and E.Christophers (2), (Introduced by Dr. R.M. McMillan) ICI Pharmaceuticals (1), Alderley Park, Macclesfield, Cheshire SK10 4TG, UK and Department of Dermatology (2), University of Kiel, FRG.

A novel monocyte-derived neutrophil-activating peptide (MONAP) has been isolated and purified to homogeneity from lipopolysaccharide and phorbol myristate acetate-stimulated human peripheral blood monocytes (Schroder et al, 1987). Structural determination of MONAP has revealed it to be a polypeptide containing 72 amino acids (Gregory et al, 1988) which has potent chemotactic and degranulating properties with apparent in vitro specificity for neutrophils. The objective of the present study was to investigate the potential in vivo inflammatory properties of MONAP in rabbit skin.

Plasma extravasation (PE) in rabbit skin was measured by the extravasation of ^{125}I-albumin as described by Williams (1979) and leukocyte infiltration was assessed histologically, in the same samples, as previously described (Aked etal, 1986). MONAP was prepared and purified to homogeneity as indicated by a single band on silver stained SDS-PAGE as described by Schroder et al, 1987.

One hr after an intradermal injection of MONAP (10pmoles) a mild infiltration of polymorphonuclear leukocytes (PMNL) was seen. Less than 2% of the cells were mononuclear. No increase in vascular permeability (PE) above a saline control injection was measured. When MONAP (1-100pmoles) was co-injected with the vasodilator PGE_2 (570pmoles) a dose-related and synergistic increase in PMNL infiltration and PE occurred. Other vasodilators (prostacyclin and vasoactive intestinal peptide) also acted synergistically with MONAP to increase vascular permeability. Kinetic studies showed that MONAP caused a linear PMNL infiltration which reached a maximum by 2hr and which was then maintained until 8hr before declining to near background levels at 24hr. When MONAP was combined with PGE_2 the PMNL profile was similar to MONAP alone except that both the rate and total numbers of PMNL achieved per site increased 5-fold. MONAP (10pmoles) alone had no effect on vascular permeability but when combined with PGE_2 it caused a transient increase which peaked at 40min and subsided by 2hr. When MONAP was co-injected with PGE_2 into rabbits made neutropenic by treatment with Nitrogen-mustard no synergistic effect on PE was observed.

These studies show that MONAP is a potent mediator of acute inflammation in rabbit skin. The synergistic interaction of MONAP with the vasodilator PGE_2 on PE and the dependence of this on circulating PMNL is similar to that previously described for other chemotactic agents (C5a, LTB_4 and FMLP). MONAP is a novel cytokine with pro-inflammatory properties and may have physiological and pathophysiological roles in health and disease.

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PAF FORMATION AND ACETYLTRANSFERASE ACTIVITY IN GUINEA-PIG EPIDERMAL CELLS

F.M. Cunningham* & M.J. Andrews, Dept. of Veterinary Basic Sciences, Royal Veterinary College, Hertfordshire, AL9 7TA.

Platelet activating factor (PAF) recovered from the lesional stratum corneum of patients with psoriasis may be released locally by the resident skin cell population since stimulated human dermal fibroblasts can produce PAF in vitro (Michel et al., 1988) and small amounts of PAF have also been detected in mixed human epidermal cell suspensions after ionophore challenge (Cunningham et al., 1987). Epidermal cell production of PAF has now been further investigated using cells prepared from guinea pig skin.

Mixed epidermal cell suspensions were prepared from guinea pig skin slices by overnight trypsinisation (0.25% trypsin 1:250; Sigma) at 4°C, separation of epidermal from dermal tissue and a further 30 min incubation with trypsin at 37°C. The cells (3-4 ml aliquots; 10^7 cells ml^{-1} in RPMI 1640 containing 0.25% bovine serum albumin) were incubated at 37°C with 2 uM ionophore A23187 and the PAF and lyso-PAF in combined cell and supernatant fractions then measured in a guinea pig platelet aggregation assay as previously described (Mallet & Cunningham 1987). Lyso-PAF was reactylated prior to quantitation in the bioassay by incubation with trifloroacetic anhydride and acetic acid (Dell & Tiller, 1986). Acetyltransferase activity in epidermal cell lysates was determined according to the method of Coeffier et al. (1986), using 40 uM lyso-PAF, 800 uM acetyl CoA and a 10 min incubation time.

The amount of PAF recovered from epidermal cells stimulated with the ionophore for 5, 15 or 30 min was no greater than that in unstimulated cells, nor was there any increase in lyso-PAF levels (Table). Epidermal cells do, however, appear to possess an acetyltransferase which formed 8.0 ± 2.4 nmol PAF/mg protein/10 min (mean ± s.e.mean, n = 4). Pre-incubation of epidermal cells with 2 uM A23187 for 5 or 60 min did not increase acetyltransferase activity (7.5 ± 3.3 nmol PAF/mg protein/10 min and 6.2 ± 2.1 nmol PAF/mg protein/10 min, mean ± s.e.mean, n = 4), although an earlier, transient, rise in activity may have occurred, as reported in thrombin stimulated platelets (Coeffier et al., 1986)

	PAF ($pg/10^7$ cells)	Lyso-PAF ($ng/10^7$ cells)
No ionophore	1180 ± 938	81 ± 67
+ ionophore (5 min)	501 ± 434	21 ± 12
(15 min)	119 ± 62	8 ± 2
(30 min)	33 ± 9	5 ± 0.5

Results are expressed as mean ± sem; n = 3

These results suggest that although epidermal cells possess an acetyltransferase which can generate PAF from lyso-PAF in the presence of acetyl CoA, incubation of these cells with the ionophore, in contrast to other cell types, does not stimulate PAF production. One possible explanation may be the absence of appropriate co-factors in intact epidermal cells.

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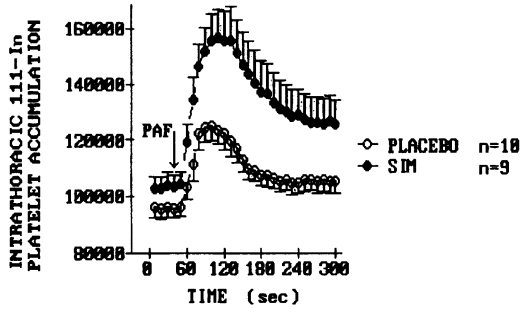
IN VIVO PLATELET HYPERAGGREGABILITY FOLLOWING CHRONIC TREATMENT WITH CYCLOSPORIN A

C. Tapparelli*, S. Sanjar, L. Mazzoni, P. Donatsch and J. Morley, Preclinical Research, Sandoz Ltd., CH-4002 Basel.

Cyclosporin A (SANDIMMUN^{R}; SIM) treatment at high doses is associated with nephrotoxicity in which renal arterioles become occluded by platelets and fibrin in the progression of arteriolopathy (Shulman et al., 1981). The haemostatic changes favouring thrombosis observed in SIM-treated patients (Vanrenterghem et al., 1985) have been correlated with SIM-dependent platelet hyperaggregability. To investigate the effect of SIM on platelet function in vivo, minipumps (Alzet, ML 1) containing SIM or vehicle were implanted s.c. in guinea-pigs (n= 9-10). After 7 days treatment, animals were anaesthetized with urethane (1.4 g/kg i.p.) and platelet responsiveness was measured as intrathoracic accumulation of ^{111}In-labelled platelets in response to i.v. PAF, ADP or collagen (Smith et al., 1988). After SIM (70 mg/kg/day), responses to ADP (0.1 mg/kg), PAF (10 ng/kg; Figure) or collagen (50 ug/kg) were enhanced. When assessed as area under the curve (AUC) for the period of observation, responses were increased by a factor of 4 for ADP, 2.6 for PAF and 2.3 for collagen. Furthermore, in animals treated with 23 or 70 mg/kg/day of SIM, (mean SIM blood levels determined by RIA according to Donatsch et al., 1981, were 195 and 299 ng/ml respectively) for 10 days, the increased platelet responsiveness to ADP or PAF was dose-related. Responses to ADP, as assessed by AUC, were increased by a factor of 1.8 at the lower dose and by 2.3 at the higher dose of SIM. Increases of 1.6 and 2.5 respectively were observed with PAF. In similar experiments using spontaneous hypertensive rats, ADP (20 ug/kg), 5-HT (50 ug/kg), collagen (50 ug/kg) and thrombin (50 IU/kg) were used to measure platelet responsiveness. In these animals, PAF, at 100 and 1000 ng/kg, was inactive (Tapparelli et al., 1987). Moreover, despite sustained SIM blood levels (900-1350 ng/ml) the aggregatory responses to ADP, 5-HT, collagen and thrombin in SIM-treated animals were similar to those observed in vehicle treated controls. Thus, chronic treatment with SIM modifies platelet responsiveness in the guinea-pig but not in the rat. It is possible that the differing effects of SIM in the guinea-pig and rat is a reflection of the absence of PAF receptors on rat platelets.



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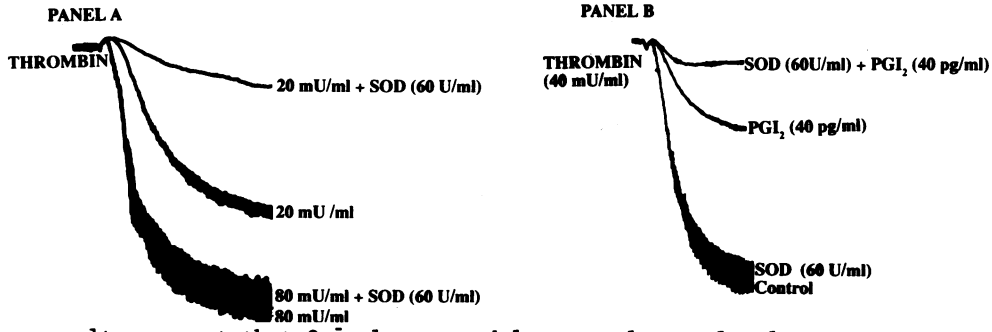
SUPEROXIDE ANIONS ENHANCE PLATELET AGGREGATION

D. Salvemini*, G. de Nucci and J.R. Vane. The William Harvey Research Institute, St Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

Superoxide anions (O_2^-) enhance platelet adhesion to a mild thrombogenic surface (Salvemini et al., 1988). However, the role of O_2^- in platelet aggregation is controversial. Handin et al. (1976) reported that O_2^- enhance platelet aggregation whereas other authors (Krinsky et al., 1981; Koufos & Sagone Jr, 1980) suggested that O_2^- served as a precursor for hydrogen peroxide which modulated platelet function. Here we report that O_2^- can enhance aggregation induced by thrombin in human washed platelets, but not in platelet rich plasma (PRP).

Human washed platelets or PRP treated with indomethacin (10 μM) were preincubated at 37°C for 2 min in a Payton dual channel aggregometer with continuous stirring at 1000 rpm. Thrombin (10-80 mU/ml) or ADP (0.5-6 μM) were added to the platelet suspension and the change in light transmission was monitored for a further 3 min. Superoxide dismutase (SOD, 60 U/ml), prostacyclin (PGI_2, 2-60 pg/ml), iloprost (20-80 pg/ml), catalase (1000 U/ml), mannitol (1 mM) or pyrogallol (5.6 μM) were added to the platelet suspension 1 min before thrombin or ADP stimulation.

SOD (60 U/ml) reduced platelet aggregation induced by low concentrations of thrombin (< 20 mU/ml) whereas it had no effect when higher concentrations of thrombin were used (Panel A). SOD also augmented prostacyclin-induced platelet inhibition in the presence of high concentrations of thrombin (Panel B). Neither catalase nor mannitol had any effect on thrombin-induced aggregation. Pyrogallol (an O_2^- generator) enhanced platelet aggregation when threshold concentrations of thrombin were used (12-16 mU/ml) and this effect was reversed by SOD. In PRP, SOD had no effect on the ADP-induced platelet aggregation, nor did it potentiate the inhibitory effect of prostacyclin.



Our results suggest that O_2^- plays a modulatory role on platelet aggregation. In addition these results indicate that potentiation by SOD of biological effects of EDRF-like substances released by blood cells is not conclusive evidence for the release of EDRF, since the simple removal of O_2^- by SOD without involvement of EDRF could be an equally important mechanism.

The William Harvey Research Institute is supported by a grant from Glaxo Group Research Limited.

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INHIBITION OF THE PHOSPHOINOSITIDE RESPONSE TO LOW CONCENTRATIONS OF CARBACHOL BY IBMX IN BOVINE TRACHEAL SMOOTH MUSCLE

I.P. Hall* & S.J. Hill, Department of Physiology & Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH.

Pharmacomechanical coupling in airway smooth muscle in response to agents such as histamine and carbachol is thought to involve the hydrolysis of phosphatidylinositol-4,5-bisphosphate to release the intracellular second messengers inositol-1,4,5-trisphosphate and diacyglycerol (Hashimoto et al., 1985). We have previously described how the formation of ^{3}H-inositol phosphates elicited by histamine can be inhibited in bovine tracheal smooth muscle by agents which elevate tissue cyclic AMP levels, or by a stable analogue of cyclic AMP (Hall & Hill, 1988: Hall et al., 1988). In this communication we report that the ^{3}H-inositol phosphate response to low concentrations of carbachol (CCh) can be inhibited by 3-isobutyl-1-methylxanthine (IBMX), rolipram (an inhibitor of the high K_m cyclic AMP selective phosphodiesterase isozyme, Reeves et al., 1987) and theophylline.

Agonist-stimulated (45 min) accumulation of total ^{3}H-inositol phosphates was measured in slices of bovine tracheal smooth muscle in the presence of 5 mM lithium chloride as described previously (Hall & Hill, 1988). Where appropriate IBMX, rolipram or theophylline were added 20 min before CCh. Other agents were added simultaneously with CCh.

CCh produced a concentration related increase in ^{3}H-inositol phosphate formation with an EC_{50} of 4.7 $\pm$ 1.3 μM (n=11). The effect of salbutamol (1μM) and IBMX (1mM) upon the CCh-induced ^{3}H-inositol phosphate was examined at both low (1μM) and high (100μM) concentrations of CCh. At these concentrations, CCh produced 17.0 $\pm$ 2.3 and 59.1 $\pm$ 9.1 (n = 10) fold increases in ^{3}H-inositol phosphate accumulation (over basal) respectively. Whereas the response to 100μM CCh was not significantly altered by IBMX (n=6) or salbutamol (n=5), the response to 1μM CCh was markedly inhibited by IBMX (88 $\pm$ 2 % inhibition, n=12, $p < 0.05$) but not by salbutamol (15 $\pm$ 8 % inhibition, n=5, $p > 0.05$). Both rolipram (0.1mM) and theophylline (1mM) also produced significant (both $p < 0.05$) inhibition of the ^{3}H-inositol phosphate response to 1μM CCh, although to a lesser extent than IBMX (rolipram = 47 $\pm$ 6 % inhibition, n=8: theophylline = 41 $\pm$ 3, n=6). Forskolin (1μM) produced a small (10 $\pm$ 2%) yet significant ($p < 0.05$, n=6) inhibition of the CCh (1μM) response but the membrane permeant analogue of cyclic AMP, 8-bromo-cyclic AMP,(1mM, n=4) was without significant effect on the response to CCh.

These results show that the ^{3}H-inositol phosphate response to low, but not high, concentrations of CCh can be inhibited by IBMX, rolipram and theophylline in bovine tracheal smooth muscle. However, in contrast to the data obtained with histamine (Hall et al., 1988), other agents which elevate or mimic the actions of cyclic AMP in this tissue had relatively small or no effect on the ^{3}H-inositol phosphate response to low concentrations of CCh.

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MODIFICATION OF METHACHOLINE- AND HISTAMINE-INDUCED STIMULATION OF IP_3 IN AIRWAY SMOOTH MUSCLE

*J.M. Langlands, J. Diamond and [1]I.W. Rodger, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada and [1]Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, U.K.

It is now widely accepted that contraction of smooth muscle is accompanied by stimulation of inositol phospholipid metabolism. In addition, it has been suggested that cyclic nucleotides may exert a controlling influence over the rate of inositol phosphate formation and hence the contractile response (Rapoport, 1986). Here we report the effects of several substances known to alter cyclic nucleotides [forskolin, sodium nitroprusside, M&B 22948 (cGMP phosphodiesterase inhibitor) and LY83583 (cGMP lowering agent)] on methacholine (Mch)- and histamine (hist)-induced stimulation of inositol 1,4,5-trisphosphate (IP_3) levels in airway smooth muscle.

Guinea-pig tracheal rings were suspended in Krebs-Henseleit solution, containing flurbiprofen (1μM) and isometric contractions measured. Agents altering cyclic nucleotides were added to the tissues 20min prior to the addition of either Mch or Hist. Tissues were freeze-clamped at fixed time points (between 0-60s) after addition of either Mch or Hist. IP_3 levels (expressed in text as pmol/mg tissue) were determined using a protein binding assay (Amersham). Values quoted are mean ± S.E. mean, n=5.

Mch (10^{-5}M) induced a transient increase in IP_3 content (basal 0.56±0.08; peak 1.08±0.09 at 10s). Hist ($3x10^{-5}$M) also increased IP_3 content transiently (basal 0.43±0.05; peak 0.84±0.06 at 10s). These transient increases in IP_3 content preceded any mechanical changes. In the presence of forskolin (10^{-6}M) Mch induced a slow rise in IP_3 content (from 0.18±0.02 to 0.27±0.04 at 30s) which continued to increase beyond 60s. This initial rise in IP_3 content occurred prior to the development of tension. Forskolin markedly slowed the generation of the Mch-induced contractile response. After forskolin Hist failed to either alter the IP_3 content or induce a contractile response.
In the presence or sodium nitroprusside (10^{-6}M) both contractile agonists induced an initial, short-lived increase in IP_3 content (Mch, from 0.42±0.06 to 1.58±0.13 at 10s; Hist, from 0.13±0.06 to 0.58±0.11 at 15s). The contractile response induced by Mch was unaffected by nitroprusside whereas that to Hist was depressed by approximately 60%. In contrast M&B 22948 (10^{-5}M) abolished both Mch- and Hist-induced IP_3 changes without affecting either the rate or magnitude of contraction.
In the presence of LY83583 (10^{-5}M), Mch induced a transient increase in IP_3 content (from 0.21±0.09 to 0.83±0.20 at 5s). In contrast, Hist increased IP_3 during the first 30s (basal 0.13±0.05; peak 0.69±0.13). LY93583 was without effect on the rate of tension development but increased the magnitudes of the Mch and Hist contractions (by 15-25%).

The results confirm the ability of Mch and Hist to induce significant, rapid elevations in IP_3 levels in airway smooth muscle. Agents that increase either cyclic AMP or cyclic GMP can markedly affect both the rate and magnitude of these IP_3 responses. Furthermore, the effects of M&B 22948 highlight a dissociation between IP_3 generation and tension generation.

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SOME MECHANICAL AND BIOCHEMICAL EFFECTS OF AH 21-132 IN GUINEA-PIG CARDIAC TISSUE AND BOVINE TRACHEALIS

J.L. Berry, J.P. Boyle, [1]K.R.F. Elliott, R.W. Foster, R.C. Small & A.J. Watt*, Department of Physiological Sciences and [1]Department of Biochemistry and Molecular Biology, University of Manchester, Oxford Road, Manchester M13 9PT, UK.

In gastro-intestinal smooth muscle and trachealis of the guinea-pig, AH 21-132 exerts non-specific relaxant effects and is a more potent inhibitor of cAMP-dependent phosphodiesterase (PDE) than cGMP-dependent PDE (Boyle et al., 1989 a,b). Whether the relaxant activity of AH 21-132 in these tissues depends upon phosphodiesterase inhibition remains to be determined.

In the guinea-pig, spontaneously-beating, paired atrial preparation, AH 21-132 (0.01-0.1mM) applied cumulatively caused a concentration-dependent increase in atrial rate. AH 21-132 (1mM) reduced atrial rate, but not to control levels. The negative chronotropic effect of AH 21-132 (1mM) was clearly associated with a transient negative inotropic effect. Theophylline (0.01-1mM) increased atrial rate but did not depress force.

In electrically-driven (diphasic, supramaximal pulses; 5ms; 5.8Hz) atria, AH 21-132 (0.01-0.1mM) increased the force of contraction while AH 21-132 (1mM) transiently reduced atrial force. Theophylline (0.01-1mM) caused a concentration-dependent increase in atrial force.

PDE isozymes were prepared from homogenates of guinea-pig cardiac ventricles and bovine trachealis using DEAE-Sepharose chromatography. Isozymes were identified by their chromatographic behaviour, substrate specificity and inhibitor (M&B 22,948; SKF 94120; Ro 20-1724) sensitivity. AH 21-132 showed selectivity as an inhibitor of the type III and IV isozymes (Table 1).

Table 1. -log molar IC50 for AH 21-132

Isozyme type	I	II	III	IV
Guinea-pig ventricle (mean ± s.e.)	4.29 ± 0.15	4.73 ± 0.20	6.83 ± 0.12	-
Bovine trachealis (mean ± s.d.)	4.15 ± 0.09	4.35 ± 0.13	-	6.10 ± 0.21

We conclude that the positive chronotropic and inotropic effects of AH 21-132 are consistent with the PDE inhibitory activity of the compound. The isozyme selectivity of AH 21-132 and differences in the functional importance of PDE isozymes amongst tissues (Torphy, 1988) may help to explain why AH 21-132 can relax airways smooth muscle at concentrations lower than those required to inhibit gastrointestinal smooth muscle (Boyle et al., 1989 a,b) or to stimulate the heart.

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AGONIST-STIMULATED CHANGES IN D-INOSITOL(1,4,5)TRISPHOSPHATE MASS IN BOVINE TRACHEAL SMOOTH MUSCLE

E.R. Chilvers*, R.A.J. Challiss, P.J. Barnes+ and S.R. Nahorski, Departments of Pharmacology and Therapeutics, University of Leicester, Leicester, LE1 9HN, and +Thoracic Medicine, National Heart and Lung Institute, London, SW3 6LY.

Inositol(1,4,5)trisphosphate (Ins(1,4,5)P_3) regulates Ca^{2+} efflux from non-mitochondrial internal stores in a wide variety of cells and has been proposed as the second messenger responsible for pharmacomechanical-coupling in airway smooth muscle (ASM) (Hashimoto et al. 1985). Although previous studies have shown agonist-stimulated increases in total [^{3}H]InsP_3 in ASM prelabelled with [^{3}H]-inositol, the predominance of Ins(1,3,4)P_3 accumulation over Ins(1,4,5)P_3, and problems with agonist-stimulated incorporation of [^{3}H]inositol into the phosphoinositides (Chilvers et al. 1988) suggest that [^{3}H]InsP_3 accumulation is a poor index of Ins(1,4,5)P_3 formation. We have therefore used a specific radioreceptor assay to quantify directly agonist-stimulated changes in D-Ins(1,4,5)P_3 concentration in ASM.

Bovine tracheal SM slices (300 x 300 µm) were preincubated for 120 min in oxygenated Krebs-Henseleit buffer at 37°C. Slices were then stimulated with carbachol (0.1 mM) or histamine (1 mM) in a final incubation volume of 300 µl. Incubations were terminated with ice-cold TCA, neutralised and D-Ins(1,4,5)P_3 assayed as described previously (Challiss et al. 1988) using a microsomal fraction prepared from adrenal cortex. To show that the binding protein is specific for Ins(1,4,5)P_3, tissue extracts were incubated with an active or heat-inactivated red blood cell membrane preparation of 5-phosphomonoesterase (Downes and Michell, 1981), in the presence or absence of [^{3}H]Ins(1,4,5)P_3, for 0-60 min prior to Ins(1,4,5)P_3 assay. 5-Phosphomonoesterase treatment resulted in identical mono-exponential decreases in [^{3}H] and cold Ins(1,4,5)P_3 levels in control and carbachol-stimulated slice extracts.

Carbachol resulted in a rapid increase in Ins(1,4,5)P_3 concentration from a resting value of 12.9 ± 0.8 to 27.0 ± 1.5 pmol/mg protein at 5 s (mean ± S.E., n = 9; $P < 0.001$). Longer periods of stimulation resulted in a prompt decrease in Ins(1,4,5)P_3 concentration, returning to pre-stimulated levels by 30 s and to below basal levels at 5 min (8.3 ± 0.5 pmol/mg protein; $P < 0.001$). The EC_{50} for carbachol-stimulated Ins(1,4,5)P_3 concentration (after 5 s stimulation) was 6.7 ± 1.5 µM. Maximal histamine stimulation resulted in a 33 ± 8% increase in Ins(1,4,5)P_3 concentration at 5 s and followed a similar biphasic time-course to that produced by the muscarinic agonist. The effects of carbachol and histamine were completely abolished by prior addition of 10 µM atropine or 10 µM mepyramine respectively.

This represents the first report of mass measurements of D-Ins(1,4,5)P_3 in ASM and its rapid formation strongly supports a primary role for this second messenger in excitation-contraction coupling in this tissue. In addition, the rapid decrease in Ins(1,4,5)P_3 concentration for periods of stimulation >5 s suggests prompt activation of the enzymes responsible for Ins(1,4,5)P_3 metabolism, since phosphatidylinositol 4,5-bisphosphate continues to be hydrolysed throughout this period (Chilvers et al. 1988).

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GLIBENCLAMIDE AND PHENTOLAMINE EACH PROVIDE SELECTIVE ANTAGONISM OF CROMAKALIM ACTING ON GUINEA-PIG TRACHEALIS

M.A. Murray*, & R.C. Small. Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester, M13 9PT.

Tested against the spontaneous tone of guinea-pig isolated trachealis, cromakalim (0.1 - 100μM), isoprenaline (1nM - 1μM) and theophylline (1μM - 1mM) each produced concentration-dependent relaxation. The maximal relaxant effects of isoprenaline and theophylline were identical to that of aminophylline. In contrast the maximal relaxant effect of cromakalim was approximately 70% of that of aminophylline.

Glibenclamide (0.1 - 10 μM) did not itself modify the spontaneous tone of the trachea, nor did it modify the relaxant actions of either isoprenaline or theophylline. In contrast glibenclamide (0.1 and 1 μM) caused a concentration-dependent rightward shift of the log concentration/effect curve of cromakalim without depressing its maximal effect. In higher concentration (10 μM) glibenclamide reduced the slope of the cromakalim log concentration/effect curve and moved the foot of the curve to a position intermediate between that observed for glibenclamide 0.1 and 1 μM. In this respect the antagonism of cromakalim by glibenclamide did not appear to be of the simple competitive type.

Phentolamine (1, 10 and 100 μM) did not itself modify the spontaneous tone of the trachea, nor did it modify the relaxant actions of either isoprenaline or theophylline. In contrast phentolamine caused concentration-dependent depression of the log concentration/effect curve of cromakalim. The antagonism of cromakalim provided by phentolamine was not related to the blockade of α_1 - adrenoceptors since prazosin (10 nM - 1 μM) failed to antagonise isoprenaline, theophylline or cromakalim.

These findings confirm the observations of other workers that glibenclamide (Quast & Cook, 1988; Wilson et al., 1988; Newgreen et al., 1989) and phentolamine (McPherson & Angus, 1989) provide selective antagonism of cromakalim acting on smooth muscle. That cromakalim does not primarily act to open the large, Ca^{2+}-dependent K^+-channels which govern the rectifying behaviour of trachealis muscle (Small & Foster, 1988) is suggested by the common ability of tetraethylammonium (TEA) (Allen et al, 1986), glibenclamide and phentolamine to antagonise cromakalim but the failure of glibenclamide and phentolamine to mimick the tonic and phasic spasmogenic activity induced by TEA (Dixon & Small, 1983).

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WHAT IS THE MECHANISM FOR PHORBOL ESTER-INDUCED RELAXATION OF GUINEA-PIG TRACHEA?

S.J. Hirst*, E.A. Smith[1], J.E. Beesley[1] and M.M. Dale, Dept. of Pharmacology, University College London, WC1E 6BT and [1]Microscopy Unit, Pharmacology Dept., The Wellcome Research Laboratories, Beckenham, Kent BR3 3BS.

Phorbol esters cause *contraction* of guinea pig (GP) lung parenchymal strip (Dale and Obianime, 1985); but *relaxation* of the trachea - both at intrinsic and agonist-induced tone (Menkes *et al.*, 1986). These qualitatively different responses are surprising in two tissues so anatomically closely related.

Menkes *et al.* implied that the relaxation was due to protein kinase C (PKC)-mediated interference with the receptor-activation coupling mechanisms associated with basal tracheal tone maintenance and contraction. Our results indicate that this explanation cannot account for the phenomenon since it was still possible to produce marked agonist-induced contractions with histamine (10 - 100μM, n>3) and carbachol (0.1 - 1μM, n>3) in the presence of a supramaximal relaxant concentration (>1μM) of the phorbol ester, 4β-phorbol dibutyrate (4β-PDBu).

There have been many reports that the trachea produces an epithelium-derived relaxant factor (EpDRF) (reviewed by Farmer, 1987). We have investigated the possibility that the 4β-PDBu-induced tracheal relaxation was due to the release of EpDRF. We found that although there was a parallel left-shift of the histamine concentration-response curve in rubbed strips as compared to unrubbed strips, confirming data by Hay *et al.* (1986), there was no shift of the 4β-PDBu concentration-response curve. Histological examination demonstrated that rubbed tissues were devoid of epithelium; whereas unrubbed preparations were intact.

We have incidentally shown that the relaxation to 4β-PDBu is *not* prevented by indomethacin (3μM) or propranolol (10μM), eliminating the possible involvement of a relaxant eicosanoid or a β-adrenergic stimulant, confirming data by Menkes *et al.* In addition we have shown that the relaxation is not due to release of histamine (acting at H_2-receptors), adenosine or a naloxone-sensitive opioid, since cimetidine (10μM), aminophylline (10μM) and naloxone (10μM) were without effect.

A possible explanation for the relaxation is that 4β-PDBu, through PKC, is able to activate the Na^+/K^+-ATPase pump, since this has been shown in GP ileum which also relaxes to phorbol esters (Sasaguri and Watson, 1988). However, it should be noted that in all tissues which relax to phorbol esters (trachea, ileum and uterus), inhibitory non-cholinergic non-adrenergic ($NANC_i$) nerves occur, and that this innervation is not present in those tissues which contract to phorbol esters (lung parenchyma and aorta). Furthermore, the concentration-response curve of the trachea to 4β-PDBu is similar to that produced by the putative $NANC_i$ transmitter, vasoactive intestinal peptide (VIP). Recent work by Belai, Burnstock and Dale has demonstrated enhanced VIP release by 4β-PDBu from a tissue known to contain $NANC_i$ nerves - the rat ileum longitudinal muscle (manuscript in preparation). It is conceivable that the relaxation of the trachea might involve PKC-mediated release of a NANC relaxant transmitter. The exact mechanism, for phorbol ester-induced relaxation in the trachea remains to be elucidated.

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K^+ STIMULATION OF THE PHOSPHOINOSITIDE PATHWAY IN GUINEA-PIG ILEUM IS PREDOMINANTLY NEURONAL IN ORIGIN AND MEDIATED BY Ca^{2+} ENTRY

J. Lai, T. Sasaguri and S.P. Watson* Dept. of Pharmacology, South Parks Road, Oxford, OX1 3QT

We have recently reported that stimulation of the phosphoinositide pathway in guinea-pig ileum by K^+ is partially inhibited by the dihyrdopyridine Ca^{2+} channel antagonist nifedipine and fully inhibited by the non-selective Ca^{2+} channel blocker, Cd^{2+} (Sasaguri and Watson, 1988). In the present report we provide further evidence that the response to K^+ is mediated by the entry of extracellular Ca^{2+} and that the majority of the response occurs in neuronal rather than smooth muscle cells.

Experiments were carried out on cross-chopped slices of guinea-pig ileum longitudinal smooth muscle prelabelled with [^{3}H]inositol as described previously (Sasaguri & Watson, 1988); the extracellular Ca^{2+} concentration was 2 mM unless stated. Atropine (1 μM) was routinely included in the incubation buffer to prevent the action of released acetylcholine. Tissues were pretreated with ω-conotoxin for 15 min before agonist additions and incubations stopped after 30 min with $CHCl_3$:MeOH:HCl (100:200:2). Total [^{3}H]inositol phosphates were extracted and analysed by Dowex anion exchange chromatography (Sasaguri & Watson, 1988). The extracellular Ca^{2+} concentration was buffered to 78 nM using 1 mM EGTA (Fabiato & Fabiato, 1979) and verified using the Ca^{2+} indicator indo-1.

Raising the K^+ concentration to 109 mM (without equimolar substitution of Na^+, see Sasaguri & Watson, 1988) increased the levels of total inositol phosphates to 248 $\pm$ 5.9 % of basal. A maximally effective concentration of scorpion toxin (12 μg/ml) evoked a similar increase in total inositol phosphates to 260 $\pm$ 13 % of basal. When these stimuli were added together the response was not significantly different to that seen with scorpion toxin alone (273 $\pm$ 13 %). Tetrodotoxin (1 μM) inhibited completely the formation of inositol phosphates by scorpion toxin (104 $\pm$ 9.9%) confirming that the response was mediated through the Na^+ channel. On the other hand, we have shown previously that tetrodotoxin has no effect on the activation of phospholipase C by K^+ (Sasaguri & Watson, 1988).

In the presence of nifedipine (10 uM) or ω-conotoxin (100 nM) the response to K^+ was decreased to 177 $\pm$ 18 and 172 $\pm$ 8.7 %, respectively. When these two Ca^{2+} antagonists were added together the response to K^+ was further inhibited to 124 $\pm$ 4.0 %. Reducing the extracellular Ca^{2+} concentration to 78 nM, thereby abolishing the Ca^{2+} gradient across the membrane, inhibited completely the response to K^+ (89.4 $\pm$ 16.3 %).

The present results provide further evidence that the response to K^+ is mediated by the entry of extracellular Ca^{2+}. Thus, the formation of inositol phosphates by K^+ is inhibited by lowering the extracellular Ca^{2+} to less than cytosolic levels or by the addition of the Ca^{2+} channel antagonists, nifedipine and ω-conotoxin. Nifedipine inhibits L-type Ca^{2+} channels which are located on both neuronal and smooth muscle cells, while ω-conotoxin inhibits N-type Ca^{2+} channels located only on neuronal tissue (Tsien et al, 1988). The ability of ω-conotoxin to inhibit the formation of inositol phosphates by K^+ provides evidence that the response takes place in neuronal tissue. This is supported further by the observation that scorpion toxin also stimulates the formation of inositol phosphates and that this is not additive with the response to K^+.

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INVESTIGATION OF THE 5-HT RECEPTORS IN GUINEA-PIG DESCENDING COLON

C.J. Grossman*, K.T. Bunce & P.P.A. Humphrey, Department of Neuropharmacology, Glaxo Group Research Ltd., Ware, Herts., SG12 0DP, U.K.

Comparison of the apparent dissociation constants for 5-HT_3 receptor antagonists in the guinea-pig longitudinal muscle myenteric plexus preparation (LMMP) with such estimates in other tissues, indicates that subtypes of 5-HT_3 receptor may exist (Butler et al., 1988). However, guinea-pig LMMP is a complicated preparation containing multiple 5-HT receptor types. Therefore, we sought a guinea-pig preparation that contains only 5-HT_3 receptors. Such a preparation has been identified in guinea-pig descending colon (GPDC), and a comparison has been made with the 5-HT_3 receptors in guinea-pig LMMP and in guinea-pig vagus nerve (GPVN).

The muscle layers of GPDC were dissected from the attached mucosa and set up in the longitudinal plane under a resting tension of 0.5-1.0g. The tissue was bathed with Krebs-Henseleit solution at 37°C and gassed with 95% O_2/5% CO_2. Contractions were measured isometrically.

5-Hydroxytryptamine (5-HT) produced a monophasic concentration-response curve with an EC_{50} value of 1.3 (1.0-1.8, 95% confidence limits)μM, while 2-methyl-5-HT and α-methyl-5-HT were full agonists with equipotent molar ratios of 3.7 (1.6-8.7) and 13.1 (6.9-24.7) respectively (5-HT=1). Estimates of antagonist affinity were measured for several 5-HT_3 receptor antagonists and the results are given in the following table.

Antagonist	Agonist	pA_2 (or pK_B) value		
		GPDC	GPI	GPVN
GR38032F	5-HT	*7.1±0.1	7.3	7.0
GR38032F	α-methyl-5-HT	7.0±0.1	-	-
Metoclopramide	5-HT	5.7±0.1	5.5	5.4
MDL72222	5-HT	6.7±0.3	6.2	6.4

GPDC=guinea-pig descending colon (this study). GPI=guinea-pig ileum (Butler et al., 1988). GPVN=guinea-pig vagus nerve (Burridge et al., 1988). n=4-9.
*pA_2 estimate, Schild slope = 1.26 (0.97-1.56); other values are calculated from a single concentration-ratio.

The contractile responses to 5-HT were resistant to antagonism by methysergide (10μM) and ketanserin (1μM) indicating the absence of 5-HT_1-like and 5-HT_2 receptors. Also, no relaxant responses were obtained to 5-HT (0.1-10μM) or 5-carboxamidotryptamine (0.1-300μM) in carbachol-contracted tissues.

Thus, in the present study only 5-HT_3 receptors, mediating contraction, were identified in GPDC and a comparison of antagonist affinity estimates (above table) indicates that the 5-HT_3 receptors in GPDC are similar to the receptors found in other guinea-pig tissues. However, the antagonists studied exhibit relatively lower affinities for the 5-HT_3 receptors in guinea-pig compared with those in rabbit and rat (Butler et al., 1988). Indeed, the existence of an "atypical" 5-HT_3 receptor in guinea-pig tissues is also suggested by the observation that phenylbiguanide (up to 10μM) was inactive both as an agonist and an antagonist in GPDC (this study) and GPVN (Burridge et al., 1988), but is an agonist at 5-HT_3 receptors in rat vagus nerve (Ireland & Tyers, 1987).

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AGEING: INFLUENCE ON THE IN VITRO MODULATION OF THE $GABA_A$ RECEPTOR BY PENTOBARBITONE AND 5α-PREGNAN-3α-OL-20-ONE

J.L.W. Yau*, D.J.K. Balfour and I.H. Stevenson, Department of Pharmacology and Clinical Pharmacology, University of Dundee, Medical School, Dundee. DD1 9SY.

Recent studies suggest that depressant barbiturates and anaesthetic steroids or their metabolites increase the apparent density of $GABA_A$ recognition sites in brain membranes via different mechanisms (Yau et al, 1988; Kirkness & Turner, 1988). In the present study, the effect of age on the modulation of the $GABA_A$ receptor complex by pentobarbitone and 5α-pregnan-3α-ol-20-one (allopregnanolone) was investigated.

Binding studies with (^{3}H)-muscimol (5 x 10^{-9}M) were performed with crude synaptosomal membrane preparations from rat cerebral cortex and hippocampus using a rapid filtration assay (Yau et al, 1988). Statistical analysis of the data was performed using an ANOVA; Duncan's test was used for post hoc analysis.

Age had no significant effect on (^{3}H)-muscimol binding to either cerebrocortical membranes or hippocampal membranes. In the presence of pentobarbitone (1 x 10^{-3}M), (^{3}H)-muscimol binding to cerebrocortical membranes was increased ($F(1,19) = 218$; $P<0.001$, Table 1) although the increase was significantly less ($F(3,19) = 4.1$; $P<0.05$) in rats aged 12-14 months ($P<0.01$) and 18-20 months ($P<0.05$) when compared with that observed in rats aged 2-3 months. Age, however, did not attenuate the increase ($F(1,16) = 254$; $P<0.001$) in (^{3}H)-muscimol binding evoked by incubating hippocampal membranes with pentobarbitone (1 x 10^{-3}M). Allopregnanolone (1 x 10^{-5}M) also increased (^{3}H)-muscimol binding to both cerebrocortical ($F(1,16) = 151$; $P<0.001$, Table 1) and hippocampal membranes ($F(1,19) = 143$; $P<0.001$) but this effect was not influenced significantly by age in either of the brain areas studied.

Table 1 Effect of pentobarbitone (1 x 10^{-3}M) and allopregnanolone (1 x 10^{-5}M) on (^{3}H)-muscimol binding in rat cerebral cortex

Age (months)	(^{3}H)-muscimol binding (fmol/mg protein)		
	Control	+ Pentobarbitone	+ Allopregnanolone
2 - 3	310 ± 19	528 ± 42	436 ± 26
7 - 8	269 ± 24	463 ± 47	354 ± 34
12 - 14	263 ± 25	380 ± 26	364 ± 33
18 - 20	279 ± 26	426 ± 47	371 ± 34

Values mean ± S.E. (n = 5-6)

It is concluded that age decreases the sensitivity of the $GABA_A$ receptor complex in cerebral cortex to pentobarbitone whereas no significant age-dependent changes in the sensitivity to allopregnanolone are observed. The study provides further evidence for the hypothesis that pentobarbitone and allopregnanolone may act at different sites on the $GABA_A$ receptor complex.

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THE EFFECT OF ARGININE AND L-N MONOMETHYL ARGININE ON THE RESPONSE OF THE BOVINE RETRACTOR PENIS TO STIMULATION OF ITS NANC NERVES

J.S. Gillespie & L. Xiaorong, Department of Pharmacology, Glasgow University, Glasgow, G12 8QQ.

Evidence suggests the EDRF is nitric oxide (NO) (Palmer et al., 1987). More recently the same group have suggested its' precursor is the guanidino group of arginine (Palmer et al., 1988). This last suggestion is based on the ability of arginine to increase the release of NO from cultured endothelial cells by bradykinin as measured both chemically and by bioassay. An even more recent reprint (Garthwaite et al., 1988) suggests cerebellar nerve cells may release EDRF through activation of N-methyl-D-aspartate receptors.

In the bovine retractor penis (BRP) the response to EDRF, to NO and to a smooth muscle inhibitory factor extracted from the tissue are similar (Gillespie and Sheng, 1988) and the response to NANC nerve stimulation and to EDRF have much in common (Gillespie, 1987) suggesting NO might be the neurotransmitter in these nerves. We have therefore tested the ability of arginine to increase and L-N monomethyl arginine (LNMMA) to block the response to field stimulation of the NANC nerves.

BRP's from the abattoir were cleaned and small strips 2 cm by approximately 2 mm in breadth removed and mounted in ring electrodes in 10 ml baths containing Krebs saline at 37 degrees centigrade and gassed with a mixture of 95% O_2 + 5% CO_2. Motor adrenergic nerves were blocked and tone raised by the addition of guanethidine 10^{-5}M. Frequency-response curves in response to field stimulation of NANC nerves were then constructed before the addition and in the presence of L arginine. Arginine in concentrations up to 3 x 10^{-4}M did not potentiate the response. Attempts were made to exhaust transmitter stores by long trains of stimulation before adding arginine. There was still no potentiation. Finally, in an attempt to deplete endogenous arginine preparations were kept in sterile Tyrodes' solution at 4 degrees centigrade for periods of up to ten days. The solution was changed daily to remove any arginine which might have leaked from the tissue. The responses of groups of tissue were studied at daily intervals from 1-10 days. Arginine did not potentiate responses at any time.

The effect of the blocking drug LNMMA was examined. It failed to inhibit the response to NANC nerve stimulation. As a control the effect of LMNA was examined on the response of fresh rabbit aortic strips to EDRF released by acetylcholine. LNMMA efficiently inhibited this response.

These results suggest arginine is not the precursor of the NANC neurotransmitter in the BRP. They do not however apply to the rat anococcygeus where LNMMA selectively inhibits NANC relaxation.

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NaF MODULATION OF ADENYLATE CYCLASE ACTIVITY IN NG108-15 CELLS INVOLVES MULTIPLE G-PROTEINS

E.Kelly*, M.Keen, P.Nobbs and J. MacDermot, Department of Pharmacology, University of Birmingham, The Medical School, Birmingham B15 2TJ.

NaF activates G-proteins and is frequently employed to provide evidence of G-protein involvement in cell signalling. In NG108-15 cells NaF produces a characteristic bell-shaped concentration-effect curve for adenylate cyclase activation (Kelly et al., 1988), activating the enzyme at lower concentrations but suppressing enzyme activity at higher concentrations. These effects of NaF may relate to interactions with different G-proteins regulating adenylate cyclase activity. Here we investigated the complex mechanisms underlying NaF modulation of adenylate cyclase.

Cells of the NG108-15 cell line (passage 16 - 25) were grown in 80cm^2 flasks to confluency and then frozen at -80^{o}C. Adenylate cyclase activity was measured in the cell homogenates (unwashed membranes) as described previously (Edwards et al., 1987). NaF activated adenylate cyclase with a maximum 3-fold increase over basal at 10mM NaF (Table 1). Higher concentrations of NaF suppressed adenylate cyclase activity. In the presence of forskolin (10μM) adenylate cyclase activity was increased by almost 20-fold. In the presence of forskolin, NaF produced a much greater increase in enzyme activity than seen in the presence of NaF alone (Table 1). In the presence of a saturating concentration of the prostacyclin receptor agonist iloprost (1 μM), NaF only produced a concentration-dependent inhibition of adenylate cyclase activity (Table 1). In another experiment we investigated the effects of NaF on iloprost-activated adenylate cyclase in the presence or absence of 100μM morphine, which produces around a 35% inhibition of enzyme activity in this cell line (Kelly et al., 1988). The NaF-dependent inhibition of 1μM iloprost-activated adenylate cyclase activity was markedly reduced in the presence of morphine (20mM NaF inhibited by 30.6 pmol cyclic AMP/min/mg protein in the presence of iloprost alone, but by only 11.9 pmol cyclic AMP/min/mg protein in the presence of iloprost and morphine; this experiment was repeated on one other occasion with the same result).

		NIL	FORSKOLIN (10μM)	ILOPROST (1μM)
NaF (mM)	1	+ 6.9	+ 68.8	- 8.7
	2	+22.1	+201.2	-16.6
	4	+35.9	+201.8	-15.7
	10	+39.7	+104.6	-29.3
	20	+27.4	- 25.9	-46.1

Table 1. Effects of NaF on adenylate cyclase in an NG108-15 cell homogenate. Values represent increases (+) or decreases (-) in enzyme activity (pmol cyclic AMP/min/mg protein). This experiment was repeated on one other occasion with the same result. Basal adenylate cyclase activities were: No addition 17.1,forskolin 301.1 and iloprost 95.5 pmol/min/mg protein.

The synergistic effect of NaF on forskolin activation is consistent with an interaction of forskolin with the catalytic subunit and NaF with Gs. On the other hand, NaF inhibits iloprost-stimulated activity, suggesting that iloprost is activating the total pool of Gs available, such that NaF can now further modulate enzyme activity only via Gi. The involvement of Gi is further suggested by morphine's partial block of the inhibitory effects of NaF. In conclusion, NaF appears to modulate adenylate cyclase activity in NG108-15 cells by interactions with Gs and Gi. We are currently examining the influence of bacterial toxins and GTP analogues to confirm these findings.

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MODULATION OF QUANTAL ACETYLCHOLINE RELEASE FROM MOTONEURONES BY ACTH REQUIRES CYCLIC NUCLEOTIDE-DEPENDENT PROTEIN KINASE

H. Chen, W.F. Dryden* and Y.N. Singh, Department of Pharmacology, University of Alberta, Edmonton, Alberta, Canada, T6G 2H7.

While efforts to elevate cyclic-AMP (cAMP) in motor nerve terminals have resulted in increases in acetylcholine (Ach) release (Dryden et al., 1988) only the presynaptic adenosine A_1 receptor present has been shown to affect adenylate cyclase, and it does so in an inhibitory manner (Chen et al., 1989). Adrenocorticotropic hormone (ACTH) has been shown to potentiate transmitter release at the frog neuromuscular junction (Johnston et al., 1983) but although ACTH enhanced noradrenaline release from sympathetic nerves has been postulated to be mediated through cAMP (Gothert and Hentrich, 1984), a link through inositol triphosphate and calcium release within the terminal is also possible (Farese et al., 1986). We have extended the original observations to a mammalian neuromuscular junction and have attempted to confirm the second messenger involved by interfering with links in the various possible sequences of events.

Mouse hemidiaphragms with a small stump of phrenic nerve attached were pinned on the Sylgard base of a 35 mm petri dish perfused with HEPES-buffered oxygenated Bretag's solution at room temperature. Intracellular recordings were made in the end-plate region using conventional glass microelectrodes. Miniature end plate potentials (MEPP's) were counted automatically using a gated counter, and the accumulated totals recorded at minute intervals on a printer. After a period of 5 consecutive minutes of stable MEPP frequency (f) used as the control period, porcine ACTH (1-39) (70-90 i.u. per mg) (Sigma) at 10 μg/ml was added to the perfusing fluid, and the MEPP(f) recorded over the ensuing 30 min. In normal Bretag's solution (1.53 mM Ca^{2+}, 3.48 mM K^+), ACTH had no effect on MEPP(f). When the experiments were repeated in the presence of 15 mM $[K^+]_o$ ($[Na^+]_o$ was reduced to maintain osmolarity) ACTH caused a sustained increase in MEPP(f) to 115% of control levels in 12 out of 20 tissues. The remaining tissues did not respond. The responses to ACTH were not affected by prior incubation with 2.5 μg/ml pertussis toxin (PTX) for 3-4 hours at 37°C, nor was it affected by the prior addition of 10 mM Li^+ to the perfusing fluid. The responses to ACTH were significantly reduced ($P<0.05$) by preincubation of the tissues with either H_7 (50 μM) or A_3 (10 μM), selective inhibitors of protein kinases.

These results confirm the presence of an ACTH receptor on phrenic nerve terminals in 60% of mice. The response does not require the mediation of a PTX sensitive G-protein, nor does it appear to involve PIP_2 breakdown. The protein kinases sensitive in common to both H_7 and A_3 are the cyclic nucleotide dependent protein kinases. It appears likely, therefore, that, as at other sites, the ACTH (1-39) receptor is linked through the G_s-protein to adenylate cyclase, leading to activation of protein kinase A.

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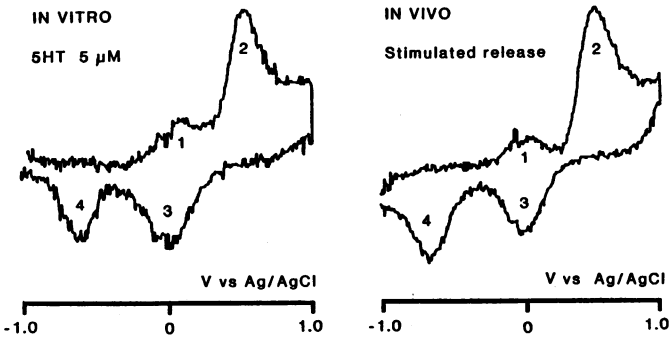
IN VIVO VOLTAMMETRIC DETECTION OF STIMULATED STRIATAL 5-HYDROXYTRYPTAMINE RELEASE AFTER PRECURSOR LOADING

J.A. STAMFORD, Z.L. KRUK & J. MILLAR, Department of Pharmacology (Physiology:JM), The London Hospital Medical College, Turner Street, London E1 2AD, U.K.

Previous studies have demonstrated the use of fast cyclic voltammetry (FCV) as a technique to monitor stimulated striatal dopamine (DA) release in vivo (Millar et al, 1985) and in vitro (Palij et al, 1988). The present report demonstrates the ability of FCV to monitor stimulated serotonin (5HT) release in real time.

Experiments were conducted in male Sprague Dawley rats (300±50g) anaesthetised with chloral hydrate (400 mg/kg). Carbon fibre microelectrodes were implanted into the striatum. Reference (Ag/AgCl) and auxiliary electrodes were placed on the skull. Electrical stimulation (50 Hz, 100 μA r.m.s., 10s train) of the median forebrain bundle (MFB) was performed via a concentric bipolar stimulating electrode. The release of monoamines in the striatum was recorded using FCV (parameters : ± 1V vs Ag/AgCl, 300 V/s scan rate) with measurements every 250 ms.

Under normal circumstances MFB stimulation causes release of DA in the striatum. However, when DA synthesis is prevented by α-methyl-p-tyrosine (AMPT) release is rapidly abolished. Subsequent administration of L-5HTP (100-400 mg/kg s.c.) after carbidopa (25 mg/kg i.p.) gives rise to release of a new compound in the striatum that is electrochemically similar to 5HT. Pretreatment of animals with L-tryptophan is ineffective. Figure 1 shows representative cyclic voltammograms of 5HT in vitro and the released compound.



Peaks 1 and 2 are oxidation waves, whereas 3 and 4 are the corresponding reduction currents. Peak 2 is the primary oxidation current, whereas peak 1 sometimes appears only as a shoulder on the main oxidation wave. The double reduction peaks make the compound readily distinguishable from DA.

Release of 5HT was dose-dependent. Following L-SHTP (200 mg/kg s.c.), peak 5HT release per stimulation was 1.53±0.14 μM (s.e.m., n=6) and the initial rate of uptake of 5HT after cessation of stimulation was 0.12±0.03 μM/s (s.e.m., n=6). The rate of uptake was reduced 42% by benztropine (20 mg/kg i.p.) indicating that significant reuptake of 5HT into DA nerves occurs under these conditions. Further studies are required to determine the site of formation and release of 5HT under this pretreatment, although it seems likely that a proportion is released as a "false transmitter" from DA neurones.

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EFFECTS OF THE 5-HT_3 RECEPTOR ANTAGONISTS, GR 38032F AND BRL 24924, ON ANXIETY IN SOCIALLY ISOLATED RATS

Alun Morinan, Division of Pharmacology, Department of Medical Sciences & Health Studies, North East London Polytechnic, Romford Road, LONDON, E15 4LZ.

Experimental evidence accumulated over the last twenty years has implicated central 5-hydroxytryptaminergic pathways in anxiety (Gardner, 1988). Further support for this hypothesis comes from the recent discovery of selective 5-HT_3 antagonists which have anxiolytic properties (Jones et al, 1988). Socially isolated rats show a heightened neophobic response which may be indicative of increased anxiety in these animals (Stanford et al, 1988). In the present study, the effects of two 5-HT_3 antagonists, GR 38032F (Jones et al, 1988) and BRL 24924 (Sanger, 1987), on the behaviour of isolated rats were investigated.

Twenty one day old male Wistar rats were housed individually for three weeks. On day 22 of isolation animals were injected (i.p) with saline (1ml/kg Controls), GR 38032F HCl (0.001-1.0 mg/kg), BRL 24924 HCl (0.001-1.0 mg/kg) or Chlordiazepoxide HCl (CDP 7.0 mg/kg). Fifty min later, each rat was placed in the dark compartment of the light/dark shuttlebox; and the latency to emerge from the dark, the number of transitions between the two compartments, the time spent in the light, and the total activity recorded over the following 5 min.

Neither GR 38032F (Table 1) nor BRL 24924 (data not shown) altered any of the four parameters measured; but CDP significantly decreased the emergence time, and increased the number of transitions (Table 1). The time spent in the light was also increased by CDP, but this just failed to reach statistical significance.

Table 1. Exploratiton in the light/dark shuttlebox after GR 38032F and CDP.

	Dose (mg/kg)	n	Transitions	Emergence(s)	Light Time(s)	Activity
GR 38032F	0.001	7	83	163	98	101
	0.01	8	57	120	52	95
	0.1	9	64	121	57	106
	1.0	9	79	123	84	99
CDP	7.0	9	300*	62*	198#	107

Each value represents the mean % of the controls (=100). The control responses (mean ± s.e.m.) were 1.8 ± 0.3 for transitions, 217 ± 15s for emergence, 12.9 ± 2.3s for light time and 763 ± 36 for activity. *$P < 0.05$ #$P > 0.05 < 0.1$

GR 38032F has been reported to increase social interaction in rats, and the activity of mice in the light side of a light/dark box (Jones et al, 1988). However this anxiolytic effect has not been confirmed in other studies using social interaction, and the elevated plus maze (Johnston & File, 1988). Further work is required to confirm the anxiolytic properties of 5-HT_3 antagonists.

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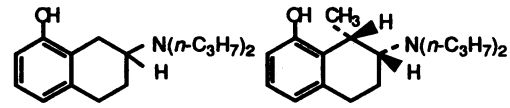
THE 8-OH-DPAT ANALOGUE CIS-(+)-1-CH_3-8-OH-DPAT,(+)ALK-3, STEREOSELECTIVELY REDUCES RAT HIPPOCAMPAL 5-HT RELEASE IN VIVO

S. Hjorth, T. Sharp, Y. Liu[1] and D. G. Grahame-Smith, MRC Unit & Univ. Dept. of Clin. Pharmacology, Radcliffe Infirmary, Oxford, England, and [1]Dept. Org. Pharmaceutical Chem., BMC, Uppsala, Sweden.

Central 5-HT autoreceptors controlling impulse flow of 5-HT neurones are generally considered to belong to the 5-HT_{1A} receptor subclass. Currently, this receptor is poorly defined because there is no selective antagonist but also there is a lack of stereoselective agonists. ALK-3 is an analogue (Figure 1) to the prototype 5-HT_{1A} ligand 8-OH-DPAT which retains the high agonist potency of the parent compound. However, in contrast to the enantiomers of 8-OH-DPAT - which display little stereoselectivity (Arvidsson et al., 1981) - ALK-3 occurs in active and inactive enantiomer forms (Arvidsson et al., 1987). Recently we reported that 8-OH-DPAT strongly reduced 5-HT release in rat hippocampus *in vivo* as assessed by brain microdialysis (Sharp et al., 1988). Here we describe the effects of the enantiomers of ALK-3 in this model.

Figure 1.



8-OH-DPAT ALK-3

Male Sprague-Dawley rats (260-330 g) were used. As previously described (Sharp et al., 1988), a dialysis probe was implanted into ventral hippocampus under chloral hydrate anaesthesia. The probe was perfused at a constant rate (1 µl/min) with artificial CSF containing the 5-HT reuptake blocker citalopram (1 µM). Perfusates were collected every 20 min and were analysed for 5-HT using HPLC-EC. (+)- and (-)ALK-3 HCl (in 0.9 % saline) were administered subcutaneously following a control period of ~3 h and measurements were continued for a further 2 h thereafter.

The baseline levels of 5-HT (approximately 0.05 pmol/perfusate) were dose-dependently decreased by (+)ALK-3 (Table I). These reductions were evident within 20 min after injection and maximum effects were seen 20-40 min later. In contrast, (-)ALK-3 (0.3 mg/kg) was inactive *per se*, and did not affect the 5-HT release-inhibitory effect of 0.3 mg/kg of (+)ALK-3 given 2 h later (n=3; not shown). A 20-min pulse of (+)ALK-3 (10 µM) via the perfusion medium did not affect 5-HT output (1 h values 92 ± 7 % of baseline; n=6), indicating that 5-HT receptors in the terminal region are not responsible for the decrease of 5-HT release seen after systemic administration of the compound. Taken together these data suggest that like 8-OH-DPAT (Hjorth & Magnusson, 1988), (+)ALK-3 is a very potent 5-HT agonist, which may inhibit terminal 5-HT release via activation of somatodendritic 5-HT, tentatively 5-HT_{1A}, autoreceptors. Importantly, and unlike 8-OH-DPAT, (+)ALK-3 is also highly stereoselective.

Table I: Effects of ALK-3 enantiomers on rat hippocampal 5-HT release 1 h after injection. Shown are the means ± s.e.m. (n=4-6), % of zero time baseline values. *p < 0.01 or less vs. controls. NT = not tested.

Dose, mg/kg SC	0	0.01	0.03	0.1	0.3
(+)ALK-3	85 ±6	76 ±4	55 ±3*	35 ±7*	36 ±3*
(-)ALK-3	85 ±6	NT	NT	NT	84 ±4

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CHARACTERIZATION OF THE RELEASE OF ENDOGENOUS 5-HYDROXYTRYPTAMINE (5-HT) AND NORADRENALINE (NA) FROM THE RAT PINEAL GLAND

Sommer M., Burns F.* & Racké K. Department of Pharmacology, University of Mainz, F.R.G.; *Department of Pharmacology, University of Glasgow, U.K.

In the pineal gland 5-HT is synthesized in pinealocytes as a precursor of melatonin. However, 5-HT is also released from the glandular cells and it has been suggested that the sympathetic nerves terminating in the pineal gland may accumulate 5-HT which then may be released together with NA (Owman, 1964; Neff et al., 1969). In the present experiments the release of endogenous 5-HT and NA was studied from the in vitro incubated pineal gland. In order to see whether both amines may be released together, the effects of electrical stimulation and of different drug treatments on the release of endogenous 5-HT and NA were compared.

Isolated pineal glands with their stalks fixed to a platinum wire were incubated in 0.5 ml Krebs solution. 5-HT and 5-hydroxyindoleacetic acid (5-HIAA) or NA released into the medium were determined by HPLC with electrochemical detection. In some experiments the tissue was stimulated electrically (pulses of 0.2 ms, 30 mA at a frequency of 5 Hz were applied 5 times for 1 min with 1 min intervalls) after 55 min (S1) and 105 min (S2). Test drugs were added to the incubation medium from the onset of incubation or 20 min before S2.

The spontaneous outflow of NA was often below the limit of detection (5-10 fmol). Electrical stimulation in the absence of drugs caused the release of 71 ± 19 fmol NA (n=4). In the presence of 10 µM nomifensine the evoked NA release was increased to 311 ± 29 fmol (n=37). Yohimbine, in addition to nomifensine, increased the release of NA in a concentration-dependent manner, maximally by 210 % at 1 µM. Prazosin up to 1 µM did not affect the release of NA. Clonidine concentration-dependently reduced the release of NA, maximally by 65 % at 1 µM. Yohimbine (1 µM) prevented the inhibitory effect of 1 µM clonidine, but not that of 10 µM clonidine.

After a 45 min period of preincubation the spontaneous outflow of 5-HT and 5-HIAA amounted to 19.2 ± 2.7 and 23.5 ± 1.2 pmol/10 min (n=6), respectively. It declined by about 60 % within 70 min of incubation. In the presence of 10 µM tryptophan the spontaneous outflow of 5-HT and 5-HIAA was 86.4 ± 3.5 and 45.0 ± 3.5 pmol/10 min (n=20) and remained nearly constant up to 2 h of incubation. Neither nomifensine, yohimbine nor clonidine affected the spontaneous outflow of 5-HT and 5-HIAA. Electrical stimulation did not affect the outflow of 5-HT and 5-HIAA, neither in the absence nor presence of tryptophan, nomifensine and/or yohimbine.

In conclusion, the release of endogenous NA from the sympathetic nerve terminating in the pineal gland is regulated by presynaptic, inhibitory α_2-adrenoceptors. This agrees with observations on the release of [^{3}H]-NA (Taranger et al., 1987). 5-HT released from the pineal gland originates almost exclusively from the pinealocytes and may not be co-released together with NA from the sympathetic nerves in significant quantities. In addition, the catabolism of 5-HT to 5-HIAA may occur mainly in an extraneuronal compartment, probably in the pinealocytes.

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ANTAGONISM BY DL-FENFLURAMINE OF EMESIS INDUCED BY APOMORPHINE, LISURIDE AND IPECACUANHA IN THE FERRET

B. Costall, R.J. Naylor and F.D. Tattersall, Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford BD7 1DP.

DL-fenfluramine has been shown to antagonise emesis induced by cisplatin in the ferret and a biochemical study of the effects of DL-fenfluramine in the area postrema indicated an action on both 5-hydroxytryptaminergic and catecholaminergic systems (Barnes et al. 1988). It was hypothesised that the antagonism of emesis induced by cisplatin may reflect a disruption of 5-hydroxytryptamine and/or dopamine function, and in the present study we investigate whether dl-fenfluramine can inhibit the emesis induced by other agents apomorphine, lisuride and ipecacuanha.

Male ferrets (1.5 to 2.0 kg) were injected subcutaneously with apomorphine and lisuride, ipecacuanha (prepared from Ipecacuanha Syrup B.P.) was administered orally. DL-fenfluramine (5 mg/kg i.p.) was injected as a 1h pretreatment. In each animal the time to onset of emesis and the numbers of vomits and retches were recorded over a 1h period (ipecacuanha and lisuride) or 30 min (apomorphine).

Table 1. Emesis induced by apomorphine (Apo.), lisuride and ipecacuanha (Ipecac.) and antagonism by dl-fenfluramine (Fenfl.)

Treatment	mg/kg	Onset of Emesis (min)	No. of vomits	No. of retches
Apo.	0.125	10± 3	2±1	12± 3
	0.25	4.5± 1	3±1	17± 4
	1.0	15± 5	1±1	5± 2
Fenl. + Apo.	0.25	29± 1*	0*	1± 1*
Lisuride	0.005	44±17	2±2	14±14
	0.001	5± 1	6±1	41± 2
	0.1	4± 1	4±1	9± 2
Fenfl. + Lisuride	0.1	>60*	0*	0*
Ipecac.	0.3	54± 6	1±1	1± 1
	0.6	26± 3	10±4	64±29
	1.2	25± 3	6±1	33±10
Fenfl. + Ipecac.	1.2	>60*	0*	0*

Each value is the mean ±S.E.M. Significant delays in the onset of emesis or reductions in vomits/retches are indicated as *P<0.05 (Mann-Whitney U test). n = 3-8.

The acute administration of dl-fenfluramine was shown to prevent the emesis induced by the dopamine agonist apomorphine, the dopamine/5-hydroxytryptamine agonist lisuride, and ipecacuanha in the ferret (Table 1). DL-fenfluramine was administered as an acute treatment since such treatments were shown to be as effective as a chronic treatment (Barnes et al. 1988) to antagonise cisplatin emesis (Tattersall, unpublished data). Whilst the mechanism by which dl-fenfluramine achieves the antagonism is uncertain, it may involve an interference with 5-hydroxytryptamine/dopamine transmission. It is concluded that dl-fenfluramine may possess a broad spectrum of antiemetic activity and be a useful tool in an elucidation of the transmitter systems mediating emesis.

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ANTAGONIST STUDIES ON THE OVERT BEHAVIOURAL EFFECTS OF MK-212 AND mCPP IN THE RAT

M. Knight and A. Fletcher (Introduced by R. J. Stephens), Department of Biomedical Research, Wyeth Research (UK), Huntercombe Lane South, Taplow, Maidenhead, Berkshire. SL6 OPH.

The aryl piperazine 5-HT agonists MK-212 (6-chloro-2-[1-piperazinyl]-pyrazine), mCPP (m-chlorophenylpiperazine) and TFMPP (m-trifluoromethylphenyl-piperazine) have been reported to exhibit agonist properties in a functional model of the 5-HT_{1C} receptor *in vitro* (Conn and Sanders-Bush, 1987). On the basis of antagonist studies Kennett and Curzon (1988) concluded that mCPP (and possibly TFMPP) induces behavioural effects in the rat which may be mediated by central 5-HT_{1C} receptors. We have extended these *in vivo* studies to examine the effects of several antagonists on behavioural changes induced by MK-212 in the rat.

Male Sprague-Dawley rats weighing 210-450 g were used in all experiments. MK-212 or mCPP were administered i.p. as solutions in saline 30 min. before placing individual animals in automated activity monitors for a period of 2 min. Immediately before being placed in the activity monitors, individual rats were observed and any overt behavioural changes noted. Both agonists dose-dependently reduced locomotor activity and induced a characteristic postural change comprising hind-limb abduction (HLA) and hunched back. In subsequent experiments the effects of antagonists on MK-212 (0.5-2.0 mg/kg i.p.) and mCPP (2.0-8.0 mg/kg i.p.) induced hypolocomotion and HLA/hunched posture were examined. The latter behaviour was scored on an arbitrary scale of 0-3 according to severity by an observer 'blind' to drug pretreatments. Antagonists were administered s.c. as solutions immediately following the i.p. administration of the agonist.

Metergoline (2.5 mg/kg), mianserin (2.0 mg/kg) and mesulergine (0.1 mg/kg) completely antagonised MK-212-induced hypolocomotion and HLA/hunched posture. These doses of metergoline and mianserin also blocked the behavioural effects of mCPP. In further studies the antagonism of MK-212 by metergoline and mianserin was shown to be dose-related. The lowest doses required to significantly antagonise MK-212 (1.5 mg/kg i.p.) induced hypolocomotion were : metergoline 0.3 mg/kg s.c. and mianserin 0.125 mg/kg s.c. The antagonism of MK-212 by mianserin was stereoselective, since (-)-mianserin (0.1-1.0 mg/kg) was inactive whereas (+)-mianserin was effective at doses of 0.1 mg/kg s.c. or greater. The antagonists alone had no significant effects on behaviour. 1-(1-naphthyl)-piperazine (1-NP), reported to be a pure antagonist of the 5-HT_{1C} receptor *in vitro* (Conn and Sanders-Bush, 1987), also blocked the behavioural effects of MK-212. However, at effective doses (0.5 mg/kg s.c. or greater), 1-NP alone significantly increased open field activity scores. Cyproheptadine (3.0 mg/kg) partially and significantly ($p < 0.01$) antagonised MK-212-induced behaviour. The following antagonists (dose in mg/kg s.c.) were inactive : ritanserin (0.5), GR 38032F (1.0), (-)propranolol (5), (-)pindolol (2) and idazoxan (1.0).

The effects of a range of 5-HT antagonists on MK-212-induced behaviour are similar to those reported by Kennett & Curzon (1988) using mCPP as agonist. Antagonists which bind with high affinity to 5-HT_{1A}, 5-HT_{1B}, 5-HT_2 or 5-HT_3 sites were ineffective whereas antagonists which bind to the 5-HT_{1C} site with high affinity clearly antagonised the overt behavioural effects of MK-212. Results with the *in vitro* 5-HT_{1C} antagonist 1-NP were less conclusive since this compound had motor stimulant effects in its own right.

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MELATONIN DOES NOT AFFECT THE DISCRIMINATIVE CUE PROPERTIES OF 5-HYDROXYTRYPTOPHAN IN THE RAT

D. Pache, P.H. Redfern, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath, BA2 7AY

In 1985, Gaffori and Van Ree demonstrated that behavioural changes evoked in the rat by administration of melatonin were inhibited by 5-hydroxytryptamine (5-HT) and mimicked by 5-HT antagonists. More recently we reported the results of experiments using a variety of agonists and antagonists at 5-HT receptor subtypes, which failed to show any significant interaction between melatonin and serotonergic mechanisms (Pache et al 1988). In an attempt to resolve this apparent contradication we have used drug discrimination techniques in order to test the ability of melatonin to interact with the cue properties of the 5-HT-precursor 5-hydroxytryptophan (5-HTP).

The method involved training rats to turn either right or left to reach hidden safe areas at the end of each side-arm of a T-maze with an electrified grid floor (0.5 mA) (Moser and Redfern 1985). Groups of 12 male rats weighing 150-200g at the start of the experiment were given carbidopa (25mg/kg ip) 60 minutes before the training session began; either saline or 5-HTP, 50mg/kg ip, was given 30 minutes before the training session began. Training sessions consisted of 8 trials one minute apart. Half the rats were required to turn left, and half right, after saline; each animal was required to turn in the opposite direction after 5-HTP. Most animals had learned the task after 40 sessions but it required 65 sessions for all the rats to reach the criterion of 80% correct responses in 10 sessions.

Once established, the discriminative cue to 5-HTP failed to generalise to melatonin, administered in doses of 1, 10 and 50mg/kg ip 30 minutes before trial. When given at the same time as 5-HTP, i.e. 30 minutes before trial, melatonin (1, 10 and 50mg/kg ip) had no effect on the learned response to 5-HTP. These results therefore further support the view that melatonin does not interact significantly with the central serotonergic system.

In a second series of experiments, in which melatonin was itself used as the cue drug, animals failed to discriminate between saline and melatonin, administered in separate experiments at doses of 1, 10 and 50mg/kg ip for up to 70 training sessions. Although the discriminative cue properties of melatonin require further investigation, these preliminary results do not provide any evidence that melatonin induces behavioural or psychotropic changes which might act as a discriminative cue.

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THE PUTATIVE 5-HT_{1A} AND 5-HT_{1B} AGONIST RU 24969 REDUCES CEPHALIC ARTERIOVENOUS SHUNTING IN THE PIG

A.H. Bom[1*], P.R. Saxena[1] & P.D. Verdouw[2]
Departments of Pharmacology[1] and Experimental Cardiology (Thorax Centre)[2], Erasmus University, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

Intracarotid infusion of 5-hydroxytryptamine (5-HT) reduces arteriovenous anastomotic (non-nutrient) blood flow (AVAF), and increases arteriolar (nutrient) blood flow (NF), resulting in a decrease in common carotid artery blood flow (CBF) in anaesthetized pigs (Saxena & Verdouw; 1982). These effects are mainly mediated by 5-HT_1-like receptors (Saxena et al., 1986). 8-OH-DPAT also reduces AVAF by an action on 5-HT_1-like receptors, whereas ipsapirone, a compound which also has a high affinity for 5-HT_{1A} recognition sites (Peroutka, 1986) is inactive (Bom et al., 1989). In order to study the possible (co)-involvement of a putative 5-HT_{1B} receptor, we have studied the effects of RU 24969, a compound with high affinities for 5-HT_{1A} and 5-HT_{1B} recognition sites, on CBF and its distribution.

Using the radioactive microsphere technique the partition of CBF into AVAF and NF was measured in 18 young Yorkshire pigs (18-37 kg) during pentobarbital anaesthesia. This was followed by the intracarotid administration of increasing doses of RU 24969. As shown in Table 1 RU 24969 caused a dose-dependent decrease in CBF and AVAF, whereas NF was mildly increased. The decrease in CBF and AVAF was slightly attenuated after pretreatment with ketanserin (0.5 mg kg^{-1}), but was markedly reduced in animals pretreated with methiothepin (1 mg kg^{-1}). Both pretreatments prevented the increase in NF by RU 24969.

TABLE 1. Effect of RU 24969 (0.3, 1.0, 3.0 and 10 µg kg min^{-1}) on CBF and its distribution in the anaesthetized pig.

Dose	Saline (n=6)			Ketanserin (n=6)			Methiothepin (n=6)		
	CBF	AVAF	NF	CBF	AVAF	NF	CBF	AVAF	NF
0.0	154±17	114±10	39±9	92±7	68±7	23±3	106±6	73±9	33±4
0.3	134±16*	94±11	39±8	76±7*	54±8*	22±3	102±8	72±9	31±4
1.0	105±11*	53±7*	52±11*	67±7*	41±5*	26±5	103±6	72±9	31±5
3.0	85±11*	29±4*	56±11*	60±8*	33±5*	27±7	95±7*	62±9*	33±5
10.0	81±11*	24±3*	58±12*	58±7*	27±6*	31±7	79±9*	42±8*	36±5

Data in ml min^{-1}; mean±s.e. mean; *, $p < 0.05$ vs. baseline

We conclude that the reduction in CBF and AVAF by RU 24969 is mainly mediated by 5-HT_1-like receptors. Due to the lack of selective antagonist for the putative 5-HT_1 receptor subtypes, it is still impossible to further characterize these 5-HT_1-like receptors in the carotid circulation of the pig.

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PARAMETRIC ANALYSIS OF TOLERANCE TO SUPPRESSANT EFFECTS OF THE 5-HT_{1A} AGONIST IPSAPIRONE ON OPERANT RESPONDING

A.J. Goudie, M. Leathley, C. Deans and M.A.I. Taylor. Department of Psychology, Liverpool University, P.O. Box 147, Liverpool L69 3BX, U.K.

Drugs acting at 5-HTlA receptors (buspirone, gepirone, ipsapirone) are anxiolytics. In clinical trials, maximal effects are obtained after chronic administration (Jacobson et al. 1985; Pecknold et al. 1985). However, most preclinical studies of these agents involve acute administration. There is therefore a need for chronic studies. We report here on effects of chronic ipsapirone in studies in which tolerance was analysed in rats with operant behaviour. In the first study, rats were run twice daily, (1000, 1600 hours) for 7 days/week in operant sessions with a DRL 15 secs schedule. Ipsapirone (10 mg/kg, i.p., 30 mins prior to sessions) initially decreased response rate to 70% of baseline and decreased number of rewards earned to 80% of baseline (ps < 0.05). Over 18 days of treatment (b.i.d.), no significant change occurred in these effects of ipsapirone.

In a second study, effects of ipsapirone on Fixed Ratio (FR20) behaviour were studied. Initially, the dose/effect curve was determined. Ipsapirone (i.p., 30 mins before sessions) suppressed responding (ED50 c. 6.0 mg/kg). Over 33 days of treatment (7.5 mg/kg daily) no tolerance developed to ipsapirone. The dose/effect curve indicated that there was no change after chronic treatment. Subsequently, animals received ipsapirone (7.5 mg/kg i.p.) three times daily, at intervals of 2.5 hours, the first injection preceding (30 mins) an operant session. Over 10 more days of treatment (t.i.d), response rates increased nonsignificantly by 13% but were still below (75% of) baseline.

In a third study, ipsapirone was administered at 7.5 mg/kg, 30 mins before FR20 operant sessions followed by two daily injections of 20 mg/kg, at intervals of 2.5 hours. Under these conditions, some tolerance developed to ipsapirone. But such tolerance was incomplete and rapidly lost.

Analysis of ipsapirone's action on FR20 responding showed that the drug is short acting. The suppressant action (to c. 50% of baseline) of a 7.5 mg/kg dose being abolished when drug was injected 4 hours before sessions. In other studies, we confirmed that tolerance develops rapidly (after one day) to hyperphagic actions of ipsapirone (Kennett et al. 1987). From these data we conclude (i) Tolerance develops at different rates to different effects of ipsapirone. (ii) Tolerance to ipsapirone's effects on responding develops only if high doses are administered frequently. Since ipsapirone is short acting, we conclude that tolerance to such effects of ipsapirone develops only when drug is continuously present. This suggests that the tolerance observed involved adaptation of 5-HT receptors. (iii) Tolerance to ipsapirone's effects on operant responding contrasts markedly with tolerance to benzodiazepines, which show rapid tolerance with infrequent injections of short-acting drugs. The difficulty reported in demonstrating tolerance to ipsapirone may be of clinical relevance, since tolerance was only observed with frequent injections of doses substantially higher than those with anxiolytic effects in animal models.

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CHRONIC CENTRAL ADMINISTRATION OF SOMATOSTATIN ANTIBODIES REDUCES COGNITIVE BEHAVIOUR IN RATS

Parkes I.R., Kenworthy R.J., Aspley S., Fone K.C.F., Marsden C.A. & Bennett G.W., Department of Physiology and Pharmacology, Medical School, Queens Medical Centre, Nottingham NG7 2UH.

Alzheimer's disease (AD) involves brain cholinergic and serotonergic deficits and selective loss of somatostatin (SOM) (Rossor & Iversen, 1986), a neuropeptide which enhances cognitive behaviour in rats (Vecsei et al., 1984). In the present study, immunization of brain SOM has been induced using chronic intracerebroventricular (i.c.v.) infusion of purified SOM antibodies (SOM-Ab) and cognitive behaviour was tested using a radial maze followed by analysis of cholinergic, serotonergic and somatostatinergic function in selected brain tissues.

Male Wistar rats (250-350g) were bilaterally cannulated (i.c.v.) and following 7 days recovery were food-deprived for 23 hr and trained on an 8 arm radial maze as described previously (Watts et al., 1981). After training rats were bilaterally injected i.c.v. twice daily for 11 days with 2x1.5 ul of either SOM-Ab or control serum. Sera IgG fractions were extracted with saturated ammonium sulphate, filtered, dialysed, shown to specifically bind SOM and diluted 1:10 with 0.9% sterile saline. During the serum injections rats were tested on the radial maze to assess memory retention on days 4, 6, 7 and 8. Basal and apomorphine (1 mg/Kg)-induced locomotor behaviour was tested on day 10. Various brain tissues (frontal, temporal and parietal cortex, hippocampus, corpus striatum, raphe and hypothalamus) were taken on day 11 for the determination of SOM levels by radioimmunoassay; 5-hydroxytryptamine (5-HT) and its major metabolite 5-hydroxyindoleacetic acid (5-HIAA) by HPLC with electrochemical detection, and choline acetyltransferase activity by radioenzymatic assay.

The initial mean number of Unrepeated Arm Choices (UAC , 7.42 ± 0.07, n=8), in the maze was not significantly altered throughout all trials with the rats injected with control serum. However, during the first two trials (on days 4 and 6) post training the mean UAC for the SOM-Ab animals was significantly reduced from 7.25 ± 0.23 to 5.43 ± 0.48 (ANOVA F ratio = 7.65, P = 0.003 comparing both within and between group differences, n=7), indicating reduced memory retention in the antibody treated rats. This reduction in UAC recovered in later trials (days 7 and 8) but remained below the control serum treated values throughout. There was no significant difference in the basal locomotor activity in the two groups and while apomorphine produced some hyperactivity in the controls it had no effect in the SOM-Ab treated rats. Furthermore, there were no significant differences between the two treatment groups in 5-HT, 5-HIAA, ChAT and SOM levels in any of the brain regions, although a small reduction in ChAT in temporal and frontal cortex was observed in the SOM-Ab animals.

These results show that immunological inactivation of cortical SOM in rats, by chronic i.c.v. administration of purified SOM-Ab, produces a significant memory deficit in comparison with animals treated with control serum. It remains to be demonstrated whether such treatment may indirectly influence cholinergic and serotonergic function, since none of the biochemical parameters determined in the present study demonstrated changes which reached significance.

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EFFECT OF NEUROTENSIN ON DOPAMINE (DA)-STIMULATED CYCLIC AMP PRODUCTION IN RAT CEREBRAL SLICES

Ford, A.P.D.W., Kendall, D.A. & Marsden, C.A., Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham, NG7 2UH.

Neurotensin (NT) is a tridecapeptide which has been widely demonstrated to inhibit dopaminergic function in mesolimbic but not nigrostriatal regions of the rat brain (for review, see Nemeroff & Cain, 1985). The mechanism by which NT influences DA receptor activation is not clear, but the neuropeptide fails to displace DA receptor ligands and is without effect on DA-stimulated cyclic AMP formation in rat striatal membrane preparations (Nemeroff et al., 1983). In this study we have examined the effect of NT on DA-stimulated cyclic AMP accumulation in "whole cell" preparations of rat brain tissue.

Cyclic AMP production was measured using the ^{3}H-adenine prelabelling method, as previously described (Donaldson et al., 1987), using cross-chopped slices (0.35 x 0.35mm) of striatal and subcortical limbic tissue from male Sprague Dawley rats. 3-Isobutyl-1-methyl-xanthine (1mM) was present in the incubation solution, as was bacitracin (28 mg/l). Antagonists (sulpiride, 50μM and propranolol, 1μM) were present in incubation tubes for 20 min before agonists. The effect of NT (1μM) was examined on the responses to 10 min incubation of both DA (300 μM) alone and with forskolin (1μM).

DA (3-300μM), in the presence of sulpiride (50μM) and propranolol (1μM), dose-dependently increased the accumulation of cyclic AMP over basal levels by 57.4 ± 7.2 and 68.7 ± 10.9% in striatal and limbic tissue respectively (n=7). Forskolin (10-1000nM), which alone stimulated cyclic AMP production in both regions, dose-dependently enhanced the DA-induced increase in a greater than additive manner. Neurotensin (see table 1) failed to influence either response.

Table 1: cyclic AMP accumulation (DPM per incubation; mean ± sem; n=3)

	basal	DA	DA + NT	Forskolin	Forskolin + DA	Forskolin + DA + NT
striatal	977±45	1564±254	1646± 94	3823±235	7310±1630	7439±1696
limbic	1257±94	1665±148	1728±151	4093±361	5564± 571	6442± 978

It can be concluded that the mechanism by which NT modifies rat brain dopaminergic function is not related directly to the inhibition or potentiation of DA-induced adenylate cyclase activity.

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TOPOGRAPHICAL DISTRIBUTION OF ANGIOTENSIN CONVERTING ENZYME IN THE COMMON MARMOSET (CALLITHRIX JACCHUS)

N.M. Barnes, B. Costall, M.E. Kelly, R.J. Naylor and T.J. Williams, Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire BD7 1DP.

Angiotensin converting enzyme (ACE) has previously been shown to be differentially distributed throughout the rodent CNS (Brownfield et al., 1982, Dabsys et al., 1988). In the present study we (a) report the topographical distribution of ACE activity in primate brain and (b) assess the ability of the ACE inhibitors captopril and SQ29852 to antagonise ACE activity using tissues taken from the common marmoset.

Male and female common marmosets (280-320g) were killed by decapitation and individual brain areas were dissected out. The tissues were homogenised in buffer (0.1 M K_2HPO_4, 0.3 M NaCl, pH 8.3). The reaction was initiated by the addition of 50 μl of homogenate to 200 μl of substrate buffer solution (final concentration 5.0 mM Hippuryl-L-histidyl-L-leucine, 0.1 M K_2HPO_4, 0.3 M NaCl) followed by incubation at 37°C for 30 mins; tubes kept on ice were used as blanks. In some experiments captopril and SQ29852 were included in the incubation media using whole brain tissue homogenates. The reaction was terminated by the addition of 50 μl 2.4M perchloric acid. Tubes were then centrifuged (15000 x g, 3 mins) and 100 μl of supernatant added to 500 μl 0.38 M NaOH. 50 μl of o-phthaldialdehyde (1% wt/vol in methanol) was added, followed 10 mins later by 50 μl 3.0 M H_3PO_4. Fluorescence was measured at an excitation wavelength of 360 nm and an emission wavelength of 500 nm. The protein content was determined using the Coomassie blue method (Bradford 1976) with bovine serum albumin as standard.

The levels of ACE activity in the brain areas studied are summarised in Table 1.

Table 1. Distribution of ACE activity in marmoset brain.

AREA	ACE ACTIVITY	AREA	ACE ACTIVITY
Nucleus accumbens	2.42±0.38	Septum	0.32±0.03
Caudate	1.30±0.60	Parietal cortex	0.27±0.03
Putamen	1.22±0.60	Temporal cortex	0.27±0.01
Hypothalamus	0.88±0.06	Entorhinal cortex	0.24±0.01
Area postrema	0.47±0.02	Amygdala	0.22±0.02
Frontal cortex	0.42±0.06	Brain stem	0.17±0.02
Hippocampus	0.36±0.07	Cerebellum	0.14±0.01

ACE activity in nmoles/mg protein/min, mean ±SEM, n = 3.

The present study demonstrates that ACE activity is differentially distributed throughout the brain of the common marmoset and, similarly to the rat, a high activity is located in limbic and striatal structures with a low activity in the cortex. ACE activity of whole brain homogenates was inhibited 90-99% by captopril and SQ29852 in a concentration dependent manner, the IC_{50} values being 62 ± 9 and 490 ± 80 nM respectively. The inhibitory effects of captopril and SQ29852 on cerebral ACE activity may be related to their ability to modify behaviour in tests of cognition (Costall et al., 1988)

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POTENTIATION BY DIHYDROPYRIDINE CALCIUM CHANNEL BLOCKERS OF PHYSOSTIGMINE-INDUCED YAWNING IN RATS

A. Bourson & P.C. Moser, Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg Cédex, France.

We have previously demonstrated that the dihydropyridine (DHP) calcium channel blocker nifedipine can potentiate yawning induced by apomorphine (Bourson *et al.*, 1986). This may reflect an interaction with either the yawning response or dopaminergic mechanisms, but the former seems more likely as other dopamine-related behaviours, such as amphetamine-induced rotation and the amphetamine discriminative stimulus, are not affected by nifedipine (Bourson *et al.*, in preparation). In order to further examine the interaction of nifedipine with yawning behaviour we have studied its effects on yawning induced by physostigmine.

Immediately following injection of either apomorphine (40 $\mu g\ kg^{-1}$ s.c.) or physostigmine (50 $\mu g\ kg^{-1}$ s.c.) male Sprague-Dawley rats (250 - 350g; Charles River, France) were placed in glass beakers and the number of yawns was recorded for the following 30 min. All experiments were carried out between 8h 30min and 12h 30min. The DHP compounds were dissolved in 50% polyethylene glycol (PEG 50%) and these solutions were protected from light. Results are presented as mean ± s.e.mean number of yawns for groups of 8 - 16 rats. Data were compared using the Mann-Whitney U-test (* $P < 0.05$).

In confirmation of previous results the DHP calcium channel blockers nifedipine (NIF), nimodipine (NIM) and nitrendipine (NIT) significantly potentiated yawning induced by apomorphine. Over the same dose-ranges these three compounds also potentiated yawning induced by physostigmine (Table 1). The non-DHP calcium channel blockers verapamil (2.5 - 10 mg kg^{-1} i.p.) and diltiazem (2.5 - 10 mg kg^{-1} i.p.) had no significant effects on yawning.

Table 1. Potentiation of apomorphine- or physostigmine-induced yawning by DHP compounds.

	Apomorphine (40 $\mu g\ kg^{-1}$ s.c.)			Physostigmine (50 $\mu g\ kg^{-1}$ s.c.)		
Dose (mg kg^{-1} i.p.)	NIF	NIM	NIT	NIF	NIM	NIT
PEG 50%	7.3 ± 0.9	9.6 ± 1.5	6.6 ± 1.2	8.7 ± 1.5	6.1 ± 1.5	4.5 ± 1.2
1.25	10.0 ± 1.9	14.9 ± 3.4	12.9 ± 2.2*	10.7 ± 2.1	12.7 ± 2.5*	7.6 ± 1.7
2.5	12.2 ± 2.1	17.1 ± 2.1*	13.2 ± 3.5	14.1 ± 2.8	7.2 ± 1.7	6.3 ± 1.1
5	12.8 ± 1.8*	11.7 ± 2.3	15.9 ± 2.0*	19.7 ± 2.1*	10.1 ± 2.8	9.4 ± 1.5*
10	15.9 ± 3.3*			15.2 ± 2.4*		

The nifedipine (10 mg kg^{-1} i.p.) potentiation of yawning induced by either physostigmine or apomorphine was completely abolished by pretreatment with atropine (2.5 mg kg^{-1} s.c.), as was yawning induced by higher doses of apomorphine or physostigmine. In contrast, sulpiride (10 mg kg^{-1} s.c.) was only able to inhibit yawning induced by nifedipine plus apomorphine whereas the effects of nifedipine plus physostigmine were unaffected (Table 2).

Table 2. Effect of sulpiride on the nifedipine potentiation of yawning.

Pretreatment	Apomorphine (40 $\mu g\ kg^{-1}$ s.c.)	Physostigmine (50 $\mu g\ kg^{-1}$ s.c.)
PEG 50%	5.0 ± 1.6	2.7 ± 1.3
Nifedipine	23.9 ± 4.2*	12.5 ± 2.9*
Nifedipine + sulpiride	5.1 ± 1.7	13.5 ± 3.2*

The inability of sulpiride to inhibit the nifedipine potentiation of physostigmine-induced yawning supports the hypothesis that this effect of DHP calcium channel blockers is not related to an effect on dopaminergic mechanisms. As *in vitro* data shows that DHP calcium channel blockers can reduce acetylcholine release, it also seems unlikely that the potentiation of yawning is related to modulation of cholinergic activity.

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THE EFFECT OF PRE- AND POST-OPERATIVE PROCEDURES ON YAWNING BEHAVIOUR IN RATS

A. Bourson & P.C. Moser, Merrell Dow Research Institute, 16 rue d'Ankara, 67084 Strasbourg Cédex, France.

During a study investigating the effects of lesions of the medial forebrain bundle (MFB) on the interaction between calcium antagonists and yawning behaviour, we found that sham-lesioned animals showed a normal nifedipine-potentiation of the yawning response to apomorphine but not to that of physostigmine (Bourson & Moser, in preparation). Ferrari & Mangiafico (1988) have previously shown that 28 day isolation can attenuate the yawning response to n-N-propylnorapomorphine and B-HT 920, and as rats were isolated for 7 days as part of our post-operative procedure the present study was undertaken to examine which aspect of the operative procedure was responsible for the selective effect we observed against physostigmine-induced yawning.

Yawning was induced in male Sprague-Dawley rats using either apomorphine (40 $\mu g\ kg^{-1}$ s.c.) or physostigmine (50 $\mu g\ kg^{-1}$ s.c.) in combination with either 50% polyethylene glycol (PEG 50%) or nifedipine (10 $mg\ kg^{-1}$ i.p.). For the observation of yawning rats were placed in glass beakers and all yawns during the subsequent 30 min period were recorded. Apomorphine or physostigmine was given immediately before the observation period; nifedipine or PEG 50% was given 30 min previously. Different groups of rats were subjected to various pretreatment schedules such as isolation for 7 days (otherwise rats were caged in groups of 5), desipramine (DMI; 25 $mg\ kg^{-1}$ i.p.) plus pentobarbital sodium (PB; 30 $mg\ kg^{-1}$ i.p.) given 7 days before testing or sham bilateral lesions of the MFB (injection of 2 µl of sterile saline containing 0.2 $mg\ ml^{-1}$ ascorbic acid at the coordinates AP = +3.8 and L = ± 1.5, both from bregma, and 8 mm below the dura according to the atlas of Pellegrino & Cushman, 1967) which included the isolation and DMI/PB pretreatments.

The sham lesioning procedure failed to prevent the nifedipine potentiation of apomorphine-induced yawning (apomorphine: 4.8 ± 1.3 yawns/30 min; apomorphine plus nifedipine: 12.6 ± 2.9; $P < 0.05$ Mann-Whitney U-test). In contrast, sham lesioning of both MFBs abolished the nifedipine potentiation of physostigmine (Table 1) as well as having a tendency to reduce physostigmine yawning itself (data not shown). Each component of the operative procedure was found to similarly abolish this effect of nifedipine on physostigmine (Table 1).

Table 1. Effect of various treatments on the nifedipine potentiation of physostigmine . All values are mean ± s.e.mean yawns/30 min for 5 - 10 rats. *P < 0.05 Mann-Whitney U-test.

Pretreatment	PEG 50%	Nifedipine
None	1.6 ± 0.9	10.6 ± 3.0*
Sham lesion of both MFB	1.1 ± 0.7	1.8 ± 0.6
DMI + PB + 7 days isolation	3.2 ± 1.4	3.0 ± 2.1
7 days isolation	1.8 ± 0.8	2.8 ± 1.5
Saline/saline	5.4 ± 1.3	11.8 ± 2.3*
DMI + PB	4.8 ± 1.5	4.3 ± 1.3

Prior stress is known to affect many behavioural and physiological parameters, and isolation stress in particular is a widely used method. Our results show that the stress associated with standard stereotaxic operative procedures is sufficient to markedly affect the pharmacological modulation of yawning behaviour and that this effect can be reproduced with the presumably milder stresses of either 7 days isolation or prior drug treatment (in this case DMI and PB). What was surprising was that this effect was selective for physostigmine-induced yawning as apomorphine-induced yawning and its potentiation by nifedipine were unaffected by the sham operation procedure. This was particularly unexpected as previous data suggests that apomorphine-induced yawning is mediated via a cholinergic system as it can be antagonised by cholinergic antagonists whereas physostigmine-induced yawning cannot be antagonised by dopamine antagonists (Holmgren & Urba-Holmgren, 1980).

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STUDIES ON THE AFFINITY OF TOBACCO ALKALOIDS AND NICOTINE METABOLITES FOR THE CENTRAL NICOTINIC RECEPTOR

G.J.B. Winch and D.J.K. Balfour, Department of Pharmacology, University Medical School, Ninewells Hospital, Dundee.

It is now widely accepted that nicotine exerts many of its psychopharmacological effects by stimulating a central nicotinic cholinoceptor which, in vitro, binds nicotine with high affinity (Clarke 1987). As part of a study designed to evaluate the possible psychopharmacological properties of other tobacco smoke constituents and the principal metabolites of nicotine, their affinities for this central nicotinic receptor have been investigated.

The test compounds were examined for their ability to displace (^{3}H)-(-)-nicotine from rat cerebrocortical membranes using the procedure described by Benwell and Balfour (1985). The incubations were performed in triplicate using at least two separate preparations. Data analysis was performed using the 'Ligand' curve-fitting programme (Munson and Rodbard, 1980).

Preliminary studies showed that (^{3}H)-(-)-nicotine bound reversibly to two sites with dissociation constants (K_d values) of 6.6 x 10^{-9}M and 5.9 x 10^{-6}M respectively (N=4 independent assays). Other nicotinic agonists (lobeline, acetylcholine, 1,1-dimethyl-4-phenylpiperazinium) also had high affinity for the acceptor site which had high affinity for nicotine (K_d = 5.9 x 10^{-9}M, 5.4 x 10^{-9}M and 5.6 x 10^{-9}M respectively) but little affinity (K_d >1 x 10^{-5}M in all cases) for the binding site which had low affinity for (-)-nicotine. Nicotinic receptor antagonists (mecamylamine; hexamethonium α-bungarotoxin) had little or no affinity for either of the binding sites (IC_{50} > 1 x 10^{-4}M). Of the compounds tested (-)-nornicotine (7.3 x 10^{-8}M), (-)-anabasine (1.5 x 10^{-7}M), N'-methyl-(-)-anabasine (2.2 x 10^{-7}M), (-)-anatabine (2.2 x 10^{-7}M), N'-methyl(-)-anatabine (5.3 x 10^{-7}M), nicotine-N-oxide (2.0 x 10^{-6}M), cotinine (2.5 x 10^{-6}M) and myosmine (4.4 x 10^{-6}M) bound to the high affinity nicotine binding site with K_d values, given in parentheses which were less than 1 x 10^{-5}M. None of the compounds had any marked affinity (K_d > 1 x 10^{-4}M) for the binding site with low affinity for (-)-nicotine. 3-Pyridylacetic acid, 3-hydroxypyridine, N'-acetyl-(-)-nornicotine and 3-hydroxycotinine had little or no affinity for either binding site (IC_{50} > 1 x 10^{-4}M). The data suggest that, with the possible exception of (-)-nornicotine, it is unlikely that compounds other than nicotine present in tobacco smoke or the principal nicotine metabolites contribute significantly to the psychopharmacological effects which are mediated by the central nicotinic receptor with high affinity for nicotine.

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EFFECTS OF NUCLEUS BASALIS LESIONS ON LEARNED SPATIAL ALTERNATION TASK IN RATS: AMELIORATION OF BEHAVIOURAL DEFICIT BY NGF

T. Kilfoil, J. Kraft, A.D. Michel, R. Whiting, C. Routledge-Ward. Institute of Experimental Pharmacology, Syntex Research, Palo Alto, CA 94304, U.S.A.

Memory impairment and degeneration of cholinergic basal forebrain neurones are characteristic of Alzheimer's disease. These cholinergic neurones innervate the cortex and hippocampus and are thought to be involved in learning and memory (Deutsch, J.A., 1971). Axotomy of these neurones in rats induce cholinergic memory deficits similar to those characteristic of Alzheimer's patients. These cholinergic deficits can be prevented by exogenous administration of NGF (Hefti, F. and Weiner, W.J., 1986; Gage, F.H., et al., 1988). To further investigate the involvement of forebrain cholinergic neurone degeneration in memory impairment we have examined the effects of nucleus basalis chemical lesions on learned spatial alternation behavior. In addition, we have studied the effects of mouse NGF in this simple memory model.

Male Wistar rats (280-320 g) were randomly assigned to five different treatment groups (n=9) and were trained to alternate in a Y-maze (Karpiak, S., 1983). After reaching an eighty percent proficiency criterion level basal forebrain lesions were made to three of the groups of rats. This involved stereotaxic injections of 5 µl of ibotenic acid (IA) (5 mg/ml) bilaterally into the nucleus basalis of Meynert (NBM). One of the remaining groups underwent sham operations and the last group received no treatment. One of the IA lesioned groups received infusions of mouse NGF (110 µg/ml, i.c.v.) another infusions of cytochrome C (250 ng/µl, c.c.v), and the remaining IA lesioned group received saline. The sham operated group also received saline infusions. Saline and drugs were infused via mini-osmotic pumps into the lateral ventricles at a rate of 0.5 µl/hr for a period of 3 weeks. All pumps had to be replaced after a two week period.

Following the three week treatment period rats were tested in the Y-maze and a number of behavioral criterion were examined to evaluate the effects of NBM lesions and NGF. Lesioned animals receiving saline or cytochrome C infusions committed significantly more total errors per day than control or sham lesioned animals ($p<0.01$) and they also took significantly longer to reach criterion ($p<0.05$) and committed significantly more perseverative errors ($p<0.05$). Lesioned animals treated with NGF committed significantly fewer perseverative errors ($p<0.05$) and the number of days to reach criterion was markedly reduced ($p<0.05$) when compared to lesion-saline treated animals. In addition, total number of errors per day was significantly decreased ($p<0.05$) when compared to lesioned, saline treated animals. There was, in fact, no significant difference between the lesioned-NGF treated group and sham lesioned controls.

In conclusion, we have established a simple, reproducible animal model to study the involvement of cholinergic neurones on learning and memory. These data demonstrate an impairment of learned spatial alternation behavior in rats followed specific chemical lesions to the NBM. NGF treatment ameliorates the lesion induced memory deficit.

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EFFECTS OF ANTICHOLINERGICS ON CHANGES IN DOPAMINE SYNTHESIS ELICITED IN RAT CNS BY ACUTE AND CHRONIC NEUROLEPTIC TREATMENT

M. Spindler, G. Stock[1] and J.D. Turner[1*] (introduced by D.N. Stephens). Psychiatric Clinic, University of Freiburg, and [1]Institute of Pharmacology, Schering AG, 1000 Berlin 65, F.R.G.

Electrophysiological and biochemical studies have shown that while acute neuroleptics stimulate firing rate and transmitter synthesis, release and metabolism in dopaminergic neurones, chronic administration (e.g. of haloperidol) leads to a reduction in the number of spontaneously active dopaminergic neurones and to blunting of the biochemical changes in response to neuroleptic challenge. One hypothesis is that a proportion of neurones, chronically disinhibited by pre- or postsynaptic dopamine (DA) receptor blockade are driven into a state of depolarisation inactivation. This phenomenon can be prevented by chronic coadministration of anticholinergics (e.g. Chiodo and Bunney, 1985 and Grace and Bunney, 1986).

In the present study the effects of acute and chronic haloperidol (HAL) and cis-flupenthixol (CFP) treatment on DA synthesis in rat striatum, estimated by measurement of DOPA accumulation following DOPA decarboxylase inhibition (NSD 1015, 100mg/kg), were assessed alone and after administration of benztropine and scopolamine. In keeping with previous studies, acute HAL (0.5mg/kg, 1 hr) stimulated DA synthesis (340% vehicle control). Chronic HAL (0.5mg/kg/day, 14, 16 or 20 days) reduced basal DA synthesis (after 16 days, 61% acute vehicle control) and the response to a HAL challenge (0.5mg/kg, 1hr) was attenuated (270% acute vehicle control after 16 days). In contrast,while acute CFP (1mg/kg, 1hr) elicited an identical stimulation of DA synthesis (342% vehicle control), no reduction of basal synthesis rate nor attenuation of response to a CFP challenge was observed after chronic CFP treatment (1mg/kg/day, 14, 16 or 20 days). However, a reduction in the response to CFP challenge was seen when HAL was the chronically administered drug.

Benztropine (25mg/kg, 2hr) and scopolamine (50mg/kg, 1hr) were without effect on DA synthesis changes elicited by acute HAL, but significantly reduced the stimulatory response to HAL challenge in 16 day HAL-treated rats (to respectively 176% and 156% of acute vehicle control). In contrast, benztropine was without effect on DA synthesis in rats acutely or chronically treated with CFP.

A possible explanation of these data is that they reflect the development of depolarisation inactivation in DAergic neurones in chronically HAL-treated rats. If so, they indicate that chronic CFP treatment does not induce it, perhaps due to its higher D1 antagonistic activity. However, the effects of anticholinergics suggest that the nature of the acetycholine-mediated feedback in striatum may also be a determining factor in the development of depolarisation block.

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EFFECTS OF REPEATED ADMINISTRATION OF THA ON THE CHOLINERGIC FUNCTIONS IN THE RAT

R. Alonso, J.P. Kan, P. Worms & P. Soubrie, Sanofi Recherche, Neurobiology Dept., rue du Pr. J. Blayac, 34082 Montpellier Cedex, France.

Tetrahydroaminoacridine (THA), a potent acetylcholinesterase (AChE) inhibitor, has been shown to be active in the treatment of Alzheimer disease (Summers et al., 1986). In rodents, chronic inhibition of AChE induced a down regulation of muscarinic receptors (Ben-Barak et al., 1981). We have thus examined the behavioural effects induced by a challenge dose of THA in rats chronically treated with this inhibitor. Possible brain adaptive changes in muscarinic M_1 and M_2 receptor subtypes were also studied.

Groups of male Sprague-Dawley rats were injected intraperitoneally (i.p.) with saline or THA during 21 days (2.5 mg/kg starting dose, with increments until 10 mg/kg). Fourty-eight hours after the last administration, rats received an acute i.p. (5 mg/kg) or oral (30 mg/kg) dose of THA and were respectively tested for chewing behaviour (Salamone et al., 1986) or for the antagonism of scopolamine (Scopo)-induced deficit in passive avoidance learning. Part of the rats were sacrificed at the end of a 48-hr wash-out period and their cortex, hippocampus and striatum assayed for M_1 and M_2 receptors using saturation or displacement experiments with [^{3}H]-Pirenzepine or [^{3}H]-N-methylscopolamine as ligands.

THA (5 mg/kg, i.p.) induced a marked increase in chewing behaviour in saline-treated rats (mean ± s.e.m. number of chewing movements; controls: 23 ± 2; THA: 151 ± 5, $p < 0.01$; Newman-Keuls test). In animals chronically administered with THA, the effects of this challenge dose of THA was significantly less pronounced (controls: 30 ± 4; THA: 100 ± 9, $p < 0.05$ as compared to saline-treated). The difference between control groups was not significant. As expected, the muscarinic antagonist, Scopo (0.5 mg/kg, i.p.) induced a dramatic decrease of the step-through latency in both groups, although the effects were less important (not significant) in THA-treated animals (Table 1). THA (30 mg/kg, p.o.) partially and almost completely reversed the Scopo-induced amnesia in the saline and THA groups, respectively (Table 1).

Table 1 Ability of a challenge dose of THA (30 mg/kg, p.o.) to antagonize the Scopo-induced deficit in passive avoidance learning in rats, chronically treated with saline or THA.

Step-through latency (s) mean ± s.e.m. (n = 10/group)					
Chronic saline			Chronic THA		
Control	Scopo	THA	Control	Scopo	THA
274 ± 16 (a)	45 ± 9 (b)	157 ± 25 (c)	286 ± 13 (d)	65 ± 6 (e)	266 ± 17 (f)

Statistical significance, $p < 0.01$: a *vs* b, a *vs* c, b *vs* c, d *vs* e, e *vs* f and c *vs* f (Newman-Keuls test).

Repeated injection of THA lowered the number of M_1 receptors in cortex (- 33 ± 4 %, $p < 0.01$, Student's-t test) and striatum (- 21 ± 4 %, $p < 0.05$). In contrast the number of M_1 sites was slightly but non significantly enhanced (+ 10 ± 5 %) in the hippocampus. In these regions, the number of M_2 receptors was not modified. In all these cases, the Kd values remained unchanged.

The results clearly indicate that the AChE inhibitor THA, when administered chronically, induced a specific down-regulation of M_1 muscarinic receptors in the cortex and in the striatum but not in the hippocampus. These adaptive changes were associated with a concomitant tolerance towards the chewing behaviour but not towards the antagonism of Scopo-induced amnesia.

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COMPARISON OF PHYSOSTIGMINE AND THA ON COGNITIVE PERFORMANCE IN YOUNG AND OLD RATS

T.K. Murray, A.J. Hunter† & A.R. Green, Astra Neuroscience Research Unit, 1 Wakefield Street, London WC1N 1PJ.

The memory impairments in patients suffering from Alzheimers disease (AD) have some similarities to those occuring in young rats treated with scopolamine (SCOP) (Dunnett 1985) and in old rats (Dunnett et al 1988). Cholinesterase inhibitors such as physostigmine (PHYSO) can reverse the deficits in AD and in these animal models. However more striking improvements in AD have recently been obtained with tetrahydroaminoacridine (THA) (Summers et al 1986). In the present experiments we compared whether THA as well as PHYSO could reverse SCOP deficits in young rats and the deficits due to aging in old rats.

Two groups of male Lister hooded rats (Harlan Olac), aged either 3 months (young) or 18 months (old) when training commenced were maintained at 90% of their free-feeding body weight. They were trained on a delayed non-matched to sample task (DNMS), in a rodent operant chamber (Sahgal 1987).

In the first experiment young rats received scopolamine HBr ($0.03 mgkg^{-1}$ s.c.) in conjunction with either THA ($1 mgkg^{-1}$ i.p.) or PHYSO ($0.1 mgkg^{-1}$ i.p.) and were compared with a SCOP + SALINE group and a control group (SALINE + SALINE). In other experiments either the young or old rats received saline and either, THA ($1 mgkg^{-1}$) or PHYSO ($0.1 mgkg^{-1}$). In all experiments at least one drug free day occured between consecutive treatments.

Table Mean % correct responses following SCOP and THA treatment in young rats

	Delay(s)					
Treatment	0	1	2	4	8	16
SAL + SAL	96.7 ±1.4	92.8 ±1.4	94.4 ±1.2	88.8 ±3.6	75.7 ±3.4	59.7 ±3.0
SAL + SCOP	87.6 ±3.0	89.1 ±2.2	76.0 ±3.7	73.6 ±4.5	65.8 ±5.5	50.5 ±5.5
THA + SCOP	94.5 ±1.9	91.6 ±1.5	88.9 ±1.6	80.5 ±2.4	67.4 ±3.2	59.8 ±3.2

Values are mean response ± s.e.mean of 8 trials at each delay (n=15, taken through each treatment in a balanced design).

Treatment with SCOP produced a significant decrease in correct responding over the test period ($p=0.05$) which was reversed by THA ($p=0.05$) (Table). PHYSO also reversed the SCOP induced deficits ($p=0.01$). Neither THA nor PHYSO alone produced improvements in performance in young rats. The performance of the old rats was impaired compared with the young animals. Both PHYSO ($0.1 mgkg^{-1}$) and THA ($1 mgkg^{-1}$) impaired their performance further.

We conclude that both THA and PHYSO reverse memory deficits due to scopolamine in young rats but that at the same dose will not improve the age-related memory impairment of old rats.

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†Present address: Smith, Kline & French Research Ltd., The Frythe, Welwyn, Herts.

CHRONIC ICV INFUSION OF HEMICHOLINIUM-3: EFFECTS ON ChAT ACTIVITY, LOCOMOTOR ACTIVITY AND PUPIL DIAMETER IN THE RAT

B. Costall, J. Coughlan, M.E. Kelly and J.M. Wilson, Postgraduate Studies in Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP

Hemicholinium-3 (HC-3), acts to inhibit acetylcholine synthesis 'in vitro' by blocking uptake of choline into cholinergic neurones. This may also provide an explanation for its effects 'in vivo' (Freeman et al., 1979).

When acutely administered, HC-3 has been shown to disrupt performance of rats in the Morris water maze (Hagan 1981). Similarly, continuous ICV infusion of HC-3 disrupts performance of Hooded Lister rats in a T-maze reinforced alternation task in a dose dependent manner (Costall et al. 1988). The present studies assess the effects of HC-3, at doses which disrupt T-maze performance, on cholineacetyltransferase (ChAT) activity, locomotor activity and pupil diameter.

HC-3 (0.01-10.0μg/day) was infused persistently into the lateral ventricles of male Hooded-Lister rats via stereotaxically located cannulae coupled to subcutaneously implanted Alzet osmotic mini pumps. Animals were tested on the T-maze (as described by Costall et al. 1988) and additionally in individual locomotor activity boxes (25x15x15cm high, with one photocell unit, counts taken/60 min) and pupil diameter measured daily. HC-3 had no effect on locomotor activity at doses below 7.5 and 10.0μg/day. These doses produced a significant reduction in the locomotor activity on day 1 where control counts/60 min were 483.6±44.2, whilst animals receiving 7.5μg/day produced counts of 341.2±36.1/60 min ($P<0.05$ Student's t-test) and those receiving HC-3 at 10.0μg/day produced counts of 312.8±34.1/60 min ($P<0.05$ Student's t-test). Pupil diameters were measured using a binocular light microscope fitted with a measuring graticule in the eye piece. Pupil diameters were unaffected throughout the test period by the infusion of HC-3 even at the highest doses used (vehicle, 7.11±0.7 x 100 μm; HC-3 7.5μg/day, 7.6±0.64 x 100 μm and HC-3 10.0μg/day, 7.4±0.50 x 100 μm ($P>0.05$ Student's t-test).

ChAT activity were determined using a modified radioenzymatic technique of Fonnum (1975). ChAT activity (pmol/min/mg protein) in rats treated with 2.5μg/day HC-3 were significantly reduced in the brain areas shown in Table 1. Reductions in ChAT activity in the hippocampus did not reach statistical significance.

Table 1. ChAT activity in vehicle and HC-3 (2.5μg/day) treated rats

Brain area	Frontal cortex	Entorhinal cortex	Hippocampus	Septum	Striatum
Control	1477±145	2732±538	1992±208	840± 71	3421±252
HC-3	1727± 95	2505±195	1231±238	1043±151	2504±174*

(P<0.05 Student's t-test)

Since ChAT activity was reduced in those animals receiving 2.5μg/day HC-3 and a good behavioural deficit was seen in the T-maze (% correct on day 9, control 83.3±2.5 and HC-3 53.3±3.3) without any overt effects on either the locomotor activity or the pupil diameter it may be concluded that the disturbance in performance in the T-maze may be a direct consequence of a disruption, by HC-3, of cerebral cholinergic function.

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CELLULAR LOCALISATION OF TYROSINE HYDROXYLASE mRNA IN RAT BRAIN AND ADRENAL MEDULLA

H Kiyama[1], J Ruth[2] and P C Emson[1]. [1]MRC Group, Dept of Neuroendocrinology, AFRC, Institute of Animal Physiology & Genetics Research, Babraham, Cambridge. [2]Molecular Biosystems, San Diego, California.

In order to localise changes in expression of the mRNA for the key catecholamine synthesising enzyme tyrosine hydroxylase we have used a novel alkaline phosphatase labelled antisense oligonucleotide probe (Jablonski et al 1986). The alkaline phosphatase labelled oligonucleotide (2 fmol/µl) was hybridised to 15µm, fresh fixed frozen tissue sections of adrenal and rat brain using a hydridisation mixture containing 4 x Standard saline citrate (4xSSC) 50% formamide, 1 x Denhardt's solution, 500 µg/ml sheared herring sperm DNA and 10% dextran sulphate. After overnight hybridisation sections were washed in four changes of 1xSSC at 55°C and then transferred into a Tris-buffered alkaline phosphatase substrate solution to visualise the TH mRNA signal. The TH mRNA signal was revealed as a purple-brown coloured product. The specificity of the signal was confirmed by competition with excess unlabelled oligonucleotide probe (200 fmol/µl) and by the susceptibility of the signal to RNase digestion (20 µg/ml). Furthermore the positive signal was only found in cells known from earlier studies with anti-tyrosine hydroxylase antibodies to contain the enzyme protein (Björklund and Hökfelt 1984).

To determine if the alkaline phosphatase labelled probe could be used in a semi-quantitative manner for measurement of the density of TH mRNA signal we compared control vehicle treated rats (n=11) with rats which had received reserpine (10 mg/kg ip) dissolved in 20% ascorbic acid (n=7). After two days, at which time the TH mRNA signal, has been reported to be maximally induced (Biguet et al 1986), rats were sacrificed and sections prepared as above. Densitometry of the alkaline phosphatase signal was carried out at 525nm using a Vickers M85 Densitometer. The results revealed that a significant increase in TH mRNA signal in locus coeruleus and substantia nigra neurones and in adrenal medulla chromaffin cells (Table 1). This data agrees with those of Biguet et al (1986) determined by northern blotting and suggests that this type of alkaline phosphatase labelled cDNA probe may allow sensitive detection of changes in TH gene expression.

Table 1 Effects of reserpine treatment on tyrosine hydroxylase mRNA

	Control (vehicle treated)	Experimental (reserpine (10mg/kg) treated)
Adrenal Medulla (chromaffin cells)	7.84±2.15 (n=200)	23.00±3.64* (n=200)
Locus coeruleus (noradrenaline neurones)	5.37±1.94 (n=200)	30.83±3.60* (n=200)
Substantia nigra (dopamine neurones)	6.64±3.13 (n=200)	9.49±4.25* (n=200)

Values are means ± SD of optical density measurements carried out on 2x2µm squares at 525nm, (n) is the number of individual cells sampled. *p<0.0001. Unpaired Students "t" test.

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EFFECTS OF pH ON AMPHETAMINE-INDUCED DOPAMINE RELEASE MEASURED BY *IN VIVO* DIALYSIS FROM RAT CORPUS STRIATUM

D.N. Thomas & R.B. Holman, Reckitt & Colman Psychopharmacology Research Unit, The Medical School, Bristol BS8 1TD.

In a number of reports using microdialysis to monitor endogenous neurotransmitter release *in vivo*, the dialysis medium is pH 6.0. This has lead to speculation about the 'non-physiological' nature of the dialysis conditions. Guan and McBride (1988) using push-pull cannulae, reported that following amphetamine (AMPH) but not elevated K+ administration, dopamine (DA) release increased 21 fold with the perfusion medium at pH 6.0, but only 3 fold at pH 7.4. We report the effects of pH on DA release from rat corpus striatum elicited by AMPH, using dialysis cannulae rather than push-pull cannulae.

Male CD rats (250-380g) were anaesthetised with chloral hydrate (400mg/kg, i.p. and 90mg/kg i.v. as required) and an *in vivo* dialysis cannulae were stereotaxically implanted into the striatum. Dialysis media containing NaCl (147mM), CaCl2 (2.3mM) and KCl (4.0mM) - pH 6.0; or as above plus 10mM Hepes - pH 7.4; was perfused through the cannula at 2.34 µl/min. Samples were collected every 15min. AMPH (2.5mg/kg in saline) was injected i.p. at the end of sample 4 and a further 8 samples were collected. Samples were analysed by HPLC-EC for DA, dihydroxyphenylacetic acid (DOPAC), homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) (Kilpatrick *et al.*, 1986). The data was analysed by two way analysis of variance.

The concentrations of endogenous DA in samples collected prior to AMPH injection did not differ significantly with respect to time of collection or pH of the dialysis medium (pH 6.0: 56.9 ± 8.17 pg/sample; pH 7.4: 58.02 ± 14.17pg/sample). Following AMPH treatment, DA release increased significantly above basal, reaching maximum output (pH 6.0: 1105.8 ± 73.75 pg/sample; pH 7.4: 1064.2 ± 64.17 pg/sample) within 30min of drug administration. AMPH-induced changes in DA release did not differ significantly at either pH. The content of DOPAC and HVA was also stable and only decline 30-45 min following AMPH. Although basal output of DOPAC did not differ significantly at pH 6.0 or 7.4, sample content of HVA was significantly less at pH 7.4. The time course and the decreases in both DOPAC and HVA content following AMPH were not significantly effected by the pH of the dialysis medium.

The present results show that there are no differences in the concentrations of endogenous DA appearing in samples collected using dialysis cannulae whether the buffer is at pH 6.0 or 7.4. However, as *in vitro* recovery of DA through these cannulae also did not differ with pH (DA 10ng/20ul: pH 6.0 - 16.72% ± 1.36 as compared with pH 7.4 - 20.45% ± 3.00), there is no reason to use dialysis buffers at other than physiological pH. The lack of agreement between results with dialysis versus push-pull cannulae emphasize that generalisations from one technique to another may not always be appropriate.

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AFFINITY OF MPTP AND MPP^+ FOR THE PRIMATE AND RODENT DOPAMINE UPTAKE SITES

N.M. Barnes, C.H.K. Cheng, B. Costall and R.J. Naylor, Department of Pharmacology, School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP, U.K.

Primates appear to be more susceptible to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) than rodents (Langston and Irwin, 1986). Both MPTP and its metabolite 1-methyl-4-phenyl-pyridinium (MPP^+) show affinity for the dopamine (DA) uptake site (Heikkila et al., 1985) which has been hypothesised as the point of entry for the neurotoxin into dopaminergic terminals. The species difference in response to the neurotoxic agents may relate to a difference in their neuronal uptake and in the present study we compare the affinity of MPTP and MPP^+ for the DA uptake site in a crude synaptosome preparation from striata of rat and marmoset.

Female Hooded-Lister rats (250-300g) and male or female common marmosets (Callithrix jacchus) (280-320g) were killed by cervical dislocation and decapitation respectively. The striatum was homogenised in a glass-teflon homogeniser in 10ml ice-cold 0.32M sucrose and centrifuged at 1000xg at 4°C for 10 min. The supernatant was recentrifuged at 48,000xg for 15 min and the pellet resuspended in ice-cold 0.27M sucrose (0.4-0.8mg protein/ml). To initiate [^{3}H]DA uptake, 100μl synaptosomal preparation was added to 900μl Krebs buffer (gassed 95% O_2, 5% CO_2) at pH7.4, containing (final concentration) 0.1μM [^{3}H]DA (50Ci/mmole), in the absence and presence of various uptake inhibitors (2.0×10^{-10}-2.0×10^{-5}M, 6 concentrations), and incubated at 37°C for 6 min. The uptake was terminated by rapid filtration through pre-wet Whatman GF/B filters, followed by washing with buffer for 6 seconds. Filter discs were counted for tritium.

Total [^{3}H]DA uptake was 1.8±0.5 and 2.4±0.2pmole/min/mg protein (mean ±SEM) in the rat and marmoset respectively. Active compounds inhibited approximately 85-90% of total [^{3}H]DA uptake, the IC_{50} values of neurotransmitter uptake inhibitors and MPTP and MPP^+ are shown in Table 1.

Table 1. IC_{50} values for drug induced inhibition of [^{3}H]DA uptake into a striatal synaptosomal preparation from rat and marmoset.

Treatment	[^{3}H]DA IC_{50} values, nM (mean ±SEM) Rat	Marmoset
MPTP	4375±658	2168±833
MPP^+	500± 58	1668±440*
GBR 12909	31± 11	93± 18*
Mazindol	40± 10	NT
Benztropine	225± 60	NT
Cocaine	513±101	NT
Desipramine	3500± 50	NT
Fluoxetine	5000±577	6833±927
Hemicholinium-3	>10,000	>10,000

n = 3-7. *P<0.05 (Dunnett's t test) NT = not tested.

These results indicate that the marmoset striatal DA uptake site is similar in substrate affinity to the rat striatal DA uptake site. MPTP inhibited at similar concentrations in both species whilst for MPP^+ and GBR12909 the affinity was only 3 fold less at the marmoset DA uptake site. Therefore the increased susceptibility to MPTP toxicity demonstrated by primates appears not to be due to an increase in affinity of MPTP or MPP^+ for the primate DA uptake site.

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THE EFFECT OF DEPRENYL ON MPTP AND MPP+ NEUROTOXICITY IN RAT NEURONAL CELL CULTURE

N.M. Barnes, C.H.K. Cheng, B. Costall and R.J. Naylor, Postgraduate Studies in Pharmacology, The School of Pharmacy, University of Bradford, Bradford, West Yorkshire, BD7 1DP, U.K.

The neurotoxicity of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) may depend on its oxidation to 1-methyl-4-phenyl-pyridinium ion (MPP^+), a reaction catalysed by monoamine oxidase B (MOAB) and the uptake of MPP^+ into the dopamine neurone via the high affinity dopamine reuptake process (Heikkila et al., 1985; Javitch et al., 1985). Recently Mytil ineou and Friedman (1988) have demonstrated that the metabolism of MPTP to MPP^+ occurs in an embryonic rat mesencephalon cell culture. Deprenyl but not Clorgyline inhibited the metabolism of MPTP and it was hypothesised that the oxidative metabolism was important to the neurotoxic potential of MPTP. In the present study we test this hypothesis by investigating the ability of deprenyl to modify the neurotoxic effects of MPTP using a rat neuronal cell culture.

Rat foe tuses (crown to rump length = 13-14mm) were taken at 15 days gestation and the brain area from the mesencephalon containing the substantia nigra was dissected out in cold phosphate buffer solution using aseptic techniques. 0.15ml dissociated cell suspension in serum supplement medium was plated in each 96 Corning cell well and incubated at 37°C, 100% humidity, 5% CO_2 for 18-20h. The medium was then replaced with serum free medium containing insulin 5μg/ml, progesterone 20nM, putrescine 100μM, selenium 30nM, transferrin 100μg/ml, L-glutamine 2mM, penicillin 100 units/ml and streptomycin 100μg/ml in equal parts of Ham F12 solution and Dulbecco's Modification of Eagle medium and incubated for 48h. Deprenyl, MPTP or MPP^+ were added to the feeding medium and incubated for a further 24h. The live and dead cells were identified using ethidium bromide/acridine orange staining and fluorescent microscopy.

Table 1. The effects of deprenyl to modify the neurotoxicity of MPTP and MPP^+ in rat foetal neuronal cell cultures.

Treatment	% Live Cells	Treatment	% Live Cells
Control	91±2	Control	84±3
MPTP 500 μM	65±2***	MPP^+ 100 μM	60±4**
MPTP 500 μM plus		MPP^+ 100 μM plus	
Deprenyl 0.1 μM	74±4*	Deprenyl 0.1 μM	66±1***
1.0 μM	76±5	1.0 μM	56±4**
10 μM	91±1	10 μM	64±3**
100 μM	81±5	100 μM	60±2***

Each value is the mean ±S.E.M., n = 3-5. Significant reductions compared to the respective control values are shown *P<0.05, **P<0.01, ***P<0.001 (Student's t-test).

Deprenyl (0.1 - 100 μM), a selective MAOB inhibitor, caused a concentration related protective effect on cell cultures against MPTP neurotoxicity, with optimum effect at 10 μM. In contrast, deprenyl failed to modify MPP^+-induced toxicity. The data supports the hypothesis that the oxidative metabolism of MPTP is a prerequisite for neurotoxicity and that the metabolite, MPP^+ may contribute to such effects.

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NICOTINE-INDUCED CATECHOLAMINE SYNTHESIS IN HIPPOCAMPUS: INVOLVEMENT OF NORADRENERGIC NEURONES WITH A REGIONAL SPECIFICITY

S.N. Mitchell, M.P. Brazell, M.M. Schugens and J.A. Gray.
MRC Brain, Behaviour and Psychiatry Research Group, Department of Psychology, Institute of Psychiatry, London SE5 8AF.

Previous in vitro biochemical studies using slice or synaptosomal preparations have indicated a sensitivity of rat hippocampal nerve terminals to relatively high concentrations of nicotine (nic.) (Balfour, 1973; Arqueros et al., 1978). We have recently shown that acute systemic administration of nic. increased dihydroxyphenylalanine (DOPA) accumulation following inhibition of amino acid decarboxylase with m-hydroxybenzylhydrazine (NSD-1015) by direct stimulation of central nicotinic receptors (Mitchell et al., 1987). We now extend these findings by investigating whether the induced response is abolished by lesions of the ascending dorsal noradrenergic bundle, and whether it is localized within areas of the hippocampus, namely CA1, CA3-4 and dentate gyrus (DG).

Bilateral 6-hydroxydopamine (6-OHDA) lesions were performed in male Sprague Dawley rats (270-280 g) (6 µg/ml 6-OHDA in 0.04% ascorbic acid; 1.5 µl over 3 min.; caudal -5.2 mm, lateral ± 1.1 mm, horizontal -5.3 mm). Vehicle injection and sham operation served as controls. Fourteen days post surgery, rats received nic. (0.8 mg/kg s.c.) or saline (1 ml/kg, s.c.) followed 15 min. later by NSD-1015 (100 mg/kg, i.p.). The animals were killed 30 min. later and the hippocampus removed. Samples were analysed by HPLC-ED. For the sub-regional study, male Sprague Dawley rats (280-300 g) received a similar nic. or saline challenge followed by NSD-1015. The hippocampus was removed, dissected and cut into 450 µm slices (Sorvall TC-2), soaked in ice cold Krebs' solution and sub-divided macroscopically into CA1, CA3-4 and DG. Samples were analysed as above.

In 6-OHDA lesioned animals noradrenaline (NA) levels were depleted by 98% and DOPA accumulation by 55% (levels of dopamine and 5-hydroxytryptamine were unaffected). In sham and vehicle controls, nic. significantly ($P<0.001$) increased DOPA accumulation, while in lesioned rats there was no observed effect (sham: saline, 57 ± 5, nic., 87 ± 5; vehicle: saline, 50 ± 3, nic., 86 ± 4; lesion: saline, 24 ± 2, nic., 20 ± 1 ng/g tissue wet weight, n=8). In intact animals, although nic. significantly increased DOPA accumulation in all sub regions, the induced response was significantly higher in the DG compared with CA3-4 and CA1 ($P<0.005$ and $P<0.001$ respectivly; t-test following a significant drug x region interaction, df 2,32, $F=3.9$, $P<0.05$).

The induced response represents an increase in NA synthesis and regional differences in the sensitivity of noradrenergic nerve terminals between the three areas may reflect their non-uniform distribution.

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THE D2 DOPAMINE AGONIST QUINPIROLE CAN EITHER MIMIC OR REDUCE THE D-AMPHETAMINE DISCRIMINATIVE CUE

Lesley Furmidge*[12], Madlen Exner[13] and David Clark[1] (Introduced by R.B. Holman)
[1]Dept. Psychology, [2]Dept. Biochemistry & Physiology, University of Reading, Reading, and [3]Inst. Psychologie, Technische Hochschule, Darmstadt, W. Germany.

Simulation of dopamine (DA) autoreceptors leads to a reduction in nerve impulse flow in DA-containing neurons, and in synthesis and release of the transmitter. Drugs which selectively activate DA autoreceptors may prove useful for the treatment of psychiatric and neurological disorders which are thought to involve excessive dopaminergic activity, e.g. schizophrenia and tardive dyskinesia. Classical D2 DA agonists, such as quinpirole, selectively activate DA autoreceptors in the low dose range. As part of an overall project to examine the behavioural significance of DA autoreceptor stimulation, we have studied the effects of quinpirole on the discriminative cue produced by d-amphetamine. This behavioural effect is thought to be mediated by enhanced activity of the mesoaccumbens DA system (Nielsen and Jepsen, 1985).

Male, Sprague Dawley rats were taught to discriminate d-amphetamine (0.5 mg/kg, i.p.) from saline (cf. Cunningham and Appel, 1986). During 25 minute training sessions, animals had to respond on the correct lever (determined by the prior injection) to obtain food reinforcement on an FR 20 schedule. In test sessions, the ability of low doses of quinpirole to reduce the d-amphetamine cue (antagonism) or higher doses to substitute for the cue (substitution) was investigated. The percentage of responses on the d-amphetamine lever (% DL responding) prior to the first reinforcement was recorded.

Substitution study (Quinpirole alone)		Antagonism study (Quinpirole + D-Amp)	
Dose (ug/kg)	% DL responding	Dose (ug/kg)	% DL responding
Saline	2.7 ± 0.6	Saline	3.1 ± 0.8
15.6	3.8 ± 2.0	12.5	85.4 ± 8.9
31.2	10.4 ± 5.5	25.0	53.2 ± 13.5 +
62.5	37.9 ± 13.9 *	50.0	83.6 ± 6.5
125.0	68.9 ± 11.1 *		
250.0	79.8 ± 12.7 *		
D-Amp	98.2 ± 0.8 *	D-Amp	97.9 ± 0.6
* Significantly different ($p<0.05$) from saline control (n=7-8).		+ Significantly different ($p<0.05$) from d-amp control (n=6-8).	

Analysis by one-way ANOVA (repeated measures design) followed by Duncan post hoc comparison of means.

Quinpirole dose dependently increased drug lever responding (ED_{50} = 91.3 ug/kg, i.p.), the highest dose completely substituting for the d-amphetamine cue. Presumably, this generalisation is due to activation of postsynaptic D2 receptors. However, when a low dose of quinpirole (25 ug/kg) was combined with d-amphetamine there was a partial antagonism of the discriminative cue. This effect is probably due to selective stimulation of DA autoreceptors by quinpirole with a consequent reduction in d-amphetamine-induced DA release.

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EFFECTS OF α_2 ADRENOCEPTOR AGONISTS ON HORMONE RELEASE FROM THE SUPERFUSED NEURAL LOBE OF THE PITUITARY OF THE RAT

A. Livingston*, K. Lederis & D. Ko. *Dept. Pharmacology, University of Bristol, Bristol, BS8 1TD and Dept. Pharmacology & Therapeutics, University of Calgary.

Binding to α_2 receptors has been demonstrated in homogenates of bovine neurohypophysis (Morris & Livingston 1984a) and autoradiographic localisation of tritiated α_2 adrenoceptor ligands has been demonstrated in the bovine neural lobe (Morris & Livingston 1984b). In this study we have investigated the effects of α_2 adrenoceptor agonists on the release of neural lobe peptides from the superfused neural lobe of the rat.

Neurointermediate lobes were rapidly dissected from decapitated adult male Wistar rats body weight 300-400g. After dissection the neurointermediate lobes were blotted on filter paper to remove the intermediate lobe. The neural lobes thus obtained were crushed onto filter paper discs and superfused as described by Livingston & Morris (1987) in a physiological solution containing NaCl 118mM, KCl 5.0mM, KH_2PO_4 41.2mM, $MgSO_4$ 1.0mM, $CaCl_2$ 0.75mM, $NaHCO_3$ 25mM Glucose 11mM and Ascorbic acid 1.0mM. After a preliminary 30 minute superfusion at a flow rate of 250µl/min at 37°C, two 5 min fractions were collected, then the neural lobes were exposed to a five minute superfusion containing 50mM K^+ (balanced by a reduction in Na^+) to stimulate hormone release, and two more five minute fractions collected, finally two more five minute fractions were collected. In each fraction the concentration of arginine vasopressin (AVP) and oxytocin (OXY) were estimated by radioimmunoasaay. The amounts released as a fraction of the total present at the start of the collection period were calculated (the fractional release constant, FRC) and the amount released during the four unstimulated fractions was compared to the amount released in the two K^+ stimulated fractions. Control experiments to examine the release in the absence of calcium ions were also performed. The effects of the two α_2 adrenoceptor agonists xylazine and clonidine on release were examined and also the α_2 adrenoceptor antagonist idazoxan.

Potassium ions stimulated the release of AVP and OXY, increasing the FRC of AVP from 0.58 ± 0.05% to 0.97 ± 0.08% and the FRC of OXY from 0.63 ± 0.06% to 0.85 ± 0.09%. In the absence of calcium ions this release was abolished with the unstimulated FRC of AVP being 0.53 ± 0.06% and the stimulated being 0.49 ± 0.09% whilst for oxytocin the values were, unstimulated 0.44 ± 0.06% and stimulated 0.38 ± 0.07%. Xylazine at a concentration of 1µM had only slight effects, but a concentration of 5µM the FRC values were, unstimulated 0.68 ± 0.10% and stimulated 0.64 ± 0.09% for AVP and unstimulated 0.68 ± 0.04% and stimulated 0.80 ± 0.06% for OXY. With clonidine the effects at 5 µM were again not particularly obvious although there appeared to be some depression of unstimulated release as well as stimulated. But at 10µM release was apparently abolished with an unstimulated FRC of 0.75 ± 0.08% and stimulated 0.84 ± 0.11% for AVP and 0.59 ± 0.04% unstimulated and 0.59 ± 0.07% stimulated for OXY.

The α_2 adrenoceptor antagonist idazoxan at 5µM had no effects on unstimulated or stimulated release for either AVP or OXY.

Thus it would appear that the α_2 adrenoceptor agonists xylazine and clonidine can suppress neural lobe peptide release but these effects are only seen at relatively high concentrations compared to their effects on noradrenaline release.

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RECEPTOR BINDING STUDIES FOR α_2-ADRENOCEPTOR AND OPIOID AGONISTS IN SELECTED AREAS OF THE SHEEP SPINAL CORD

S.A. Brandt & A. Livingston, Department of Pharmacology, Medical School, Bristol, BS8 1TD.

Autoradiographic studies on the binding of [^{3}H] clonidine, [^{3}H] DAGO and [^{3}H]DPDPE have demonstrated discrete α_2-adrenergic and opioid binding sites in the sheep spinal cord. [^{3}H] clonidine, defining α_2-adrenergic binding sites, was bound selectively in laminae I, II, X and intermediolateral bundle (IML) as well as diffusely throughout the ventral horn (VH) of the grey matter. Opioid binding sites using [^{3}H] DAGO (μ receptor agonist) and [^{3}H] DPDPE (δ receptor agonist) were located in lamina II and laminae I and II respectively (Bouchenafa and Livingston, 1987, 1989).

In order to quantify the binding sites in the specific regions demonstrated autoradiographically the appropriate areas were dissected from the sheep spinal cord and saturation binding studies performed using the appropriate ligands. For α_2-adrenergic receptor binding studies 4 regions were dissected out; laminae I and II, lamina X, IML and VH. Varying concentrations of [^{3}H] clonidine were used to measure total binding and cold idazoxan was used to measure non-specific binding. For opioid binding, blocks containing laminae I and II were used, [^{3}H] DAGO and unlabelled naloxone measured μ receptor binding and [^{3}H] DPDPE and unlabelled ICI 154129 measured δ receptor binding. For each ligand and region both maximum binding site number (B_{max}) and afffinity (K_D) were calculated.

The values for α_2-adrenergic receptor binding were as follows:

laminae I and II	B_{max} = 268 ± 13fmol/mg protein	K_D = 1.66 ± 0.35nM (n=5)
lamina X	B_{max} = 151 ± 16fmol/mg protein	K_D = 2.96 ± 0.60nM (n=5)
IML	B_{max} = 209 ± 23fmol/mg protein	K_D = 2.71 ± 0.61nM (n=4)
Ventral horn	B_{max} = 122 ± 7 fmol/mg protein	K_D = 2.97 ± 0.43nM (n=5)

For opioid binding the values were:

laminae I and II μ(DAGO)	B_{max} = 57 ± 6fmol/mg protein	K_D = 2.93 ± 0.28nM (n=5)
laminae I and II δ(DPDPE)	B_{max} = 39 ± 10fmol/mg protein	K_D = 3.22 ± 0.81nM (n=4)

Thus in the sheep spinal cord there was a variation in the maximum number of binding sites of α_2-adrenergic ligands in the different regions studied, which agrees well with the intensities of radioligand binding observed in the autoradiographs. The K_D values were similar. There were fewer opioid (μ and δ) binding sites than α_2-adrenergic sites in laminae I and II, where the opioid binding sites are essentially located. There appeared to be slightly more μ binding sites than δ although K_D values were similar.

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OPIOID RECEPTOR SELECTIVITY OF THE (+) AND (-) ENANTIOMERS OF SPIRADOLINE AND PD117302

K.G. Meecham, S.J. Boyle, J.C. Hunter and J. Hughes. Parke Davis Research Unit, New Addenbrookes Hosp. Site, Hills Rd, Cambridge. CB2 2QB.

Optical resolution of the kappa selective opioid U62066 (spiradoline) (Lahti et al, 1985) demonstrates that the kappa agonist activity is derived from the (-)enantiomer while the (+)enantiomer displays mu agonist activity (Vonvoigtlander & Lewis, 1988). The present study compares the opioid binding affinities obtained for the enantiomers of the phenylacetamide U62066 with those of the benzothieneacetamine PD117302 (Clark et al, 1988). Receptor selectivity and efficacy for each enantiomer was also investigated using a smooth muscle bioassay.

Homogenate binding was performed using guinea-pig forebrain membranes, with the radioligands [^{3}H]U69593 and [^{3}H][D-Ala^2, MePhe^4, Glyol^5]enkephalin used to label kappa and mu opioid receptor subtypes respectively (non-specific binding defined by naloxone). The isolated field stimulated guinea-pig ileum was used for efficacy studies, with pA_2 values obtained for the non-selective opioid antagonist naloxone (Nx) and the kappa-selective antagonist norbinaltorphamine (norBNI).

Binding analysis confirmed the kappa selectivity of both U62066 and PD117302. Analysis of the (-)enantiomer of U62066 (U63460) revealed a high kappa receptor affinity and a mu/kappa ratio of 340. In contrast, the (+)enantiomer (U63639) displayed not only a low kappa affinity but also a 140-fold mu receptor selectivity. Analysis of the enantiomers of PD117302 revealed a greater kappa selectivity for the (-)enantiomer (PD123475) (mu/kappa ratio: 1060), while equally low affinities were observed at the mu and kappa receptors for the (+)enantiomer (PD123497).

Compound	kappa Ki (nM)	mu Ki (nM)	m/k ratio	Nx pA_2	NorBNI pA_2
(±)PD117302	0.5 ± 0.05	400 ± 90	800	7.8 ± 0.09	-
(-)PD123475	0.39 ± 0.09	414 ± 50	1060	7.4 ± 0.1	10.2 ± 0.04
(+)PD123497	420 ± 85	280 ± 24	0.67	7.4 ± 0.03	9.7 ± 0.15
(±)U62066	0.35 ± 0.06	38 ± 4.8	108	7.6 ± 0.09	-
(-)U63640	0.31 ± 0.07	84 ± 5.7	270	7.7 ± 0.1	9.6 ± 0.13
(+)U63639	1360 ± 220	9.8 ± 0.4	0.007	8.2 ± 0.08	7.1 ± 0.18

Values represent mean ± SEM of between 4 and 9 experiments.

Efficacy studies with the opioid antagonists showed that the (-)enantiomers of both ligands and the (+)enantiomer of PD117302, displayed profiles consistent with those of kappa agonists. The (+)enantiomer of U62066 however displayed a profile consistent with that of a mu agonist.

Thus, the kappa receptor agonist effects of both U62066 and PD117302 were shown to be derived predominantly from the (-)enantiomers of each compound. In contrast, the (+)enantiomers gave considerably different profiles with (+)U63639 showing clear mu agonist activity while no detectable mu agonist activity was observed for (+)PD123497. This study clearly demonstrates the importance of being aware of the pharmacological activity of individual isomers when investigating racemic compounds.

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THE EFFECT OF OPIATE AGONISTS AND ANTAGONISTS ON TRANSIT IN THE DISTAL COLON OF THE RAT

I.T. Collie[†], P.W. Dettmar, E.C. Leach and J.A.H. Lord[‡], Department of Biology, Reckitt & Colman plc, Dansom Lane, Hull, HU8 7DS and [‡]Reckitt & Colman Psychopharmacology Unit, The Medical School, University of Bristol, University Walk, Bristol.

The antimotility effect of morphine and other opiates on the gastrointestinal tract has been extensively studied in rodents, although little attention has been directed towards effects on the large intestine. Morphine was found to inhibit colonic bead expulsion in the mouse following i.c.v. and i.p. administration (Jacoby and Lopez, 1984) and further studies have characterised the central opioid receptor subtypes mediating this effect as μ and δ (Raffa et al., 1987). We have investigated the effects of several opiate agonists and antagonists on the transit of a marker in the distal colon and rectum of the rat.

The method adopted was to administer per rectum a coloured agar bolus (0.5 ml) 5 cm from the anus and to determine the time of emergence of the coloured pellet. Groups of rats (male Sprague_Dawley, 150-250 g, n=5-10) were pretreated with drugs or saline (s.c. 5 $mlkg^{-1}$) and the agar bolus was introduced 30 minutes later. The percentage of rats in each group that did not eject the pellet within a further 30 minutes was determined; ED50 values (doses which cause 50% animals to exceed 30 minutes) and 95% confidence limits were calculated according to the method of Litchfield and Wilcoxon (1949).

Table 1 Effect of opiates on transit in the distal colon of the rat

Drug	ED50 $mgkg^{-1}$ s.c.
Morphine	0.22 (0.13-0.34)
Fentanyl	0.004 (0.002-0.006)
Loperamide	2.30 (1.40-3.70)
Ethylketocyclazocine	0.36 (0.23-0.58)
Bremazocine	1.10 (0.61-1.98)
U69593	7.80 (4.6-13.3)

The predominantly μ agonists morphine, fentanyl and loperamide displayed antitransit effects which were antagonised by 0.3 $mgkg^{-1}$ s.c. naltrexone (non-selective antagonist) or 1 $mgkg^{-1}$ s.c. RX8008M (μ, δ antagonist; Smith, 1987). Neither antagonist when administered alone had a significant effect on transit time. The predominantly k agonists ethylketocyclazocine, bremazocine and U69593 inhibited transit at doses which also caused diuresis, which was not observed with the μ agonists; both effects were antagonised by prior treatment with naltrexone (0.3 $mgkg^{-1}$ s.c.), but not by RX8008M (1 $mgkg^{-1}$ s.c.). These observations indicate that k agonists inhibit transit in the distal colon by a mechanism mediated by k receptors. Previous observations have shown that k receptor activation does not affect small intestinal transit in the rat (Gambino et al., 1983; Lord, unpublished observations). Further work is required to identify the central and/or peripheral mechanisms responsible for the observations reported here in the rat colon and rectum.

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POTENTIATION OF MORPHINE-INDUCED ANTINOCICEPTION IN MICE BY CENTRALLY- BUT NOT PERIPHERALLY-ACTING HISTAMINE H_1 ANTAGONISTS

Patricia Freeman & Gillian Sturman, Division of Pharmacology, Dept. of Medical Sciences & Health Studies, North East London Polytechnic, Romford Rd., E15 4LZ.

Several lines of evidence suggest the involvement of histamine in the mediation o pain sensation (Dews & Graham,1946, Rumore & Schlichting,1986), without, however, demonstrating whether these actions were central and/or peripheral. Reported interactions between opioid drugs and histamine antagonists (Bluhm et al., 1982) would suggest a central role for histamine and the recent development of H1 antihistamines devoid of central activity (Neimegeers et al.,1982) facilitates the investigation of these phenomena.

Groups of ten female albino mice (Triangle,Devon) were pretreated with antihistamine or vehicle by s.c. injection. At predetermined times thereafter the mice were tested for baseline response to two nociceptive stimuli; tail pinch response to an artery clip (Bianchi & Francescini,1954) and response to a hot plate at 55°C,(Eddy & Leimbach,1953). For the tail pinch, animals failing to respond within 30 s were recorded as demonstrating analgesia and for the hot plate latency of response was recorded in s. Animals were then dosed s.c. (at a second site) with morphine sulphate or saline and retested at 30, 60 & 90 min. Tail pinch results are expressed as % analgesia in each group and latencies as median reaction times.

Table 1 shows that mepyramine, a CNS-penetrating H1 antagonist, but not astemizole, an H1 antagonist with solely peripheral effects, produced a dose-related potentiation of antinociception induced by low doses of morphine in the tail pinch but not the hot plate test. At the doses employed neither compound alone showed sedative or antinociceptive activities.

Table 1

Pretreatment ($mg.kg^{-1}$ s.c.)	Morphine dose ($mg.kg^{-1}$ s.c.)	Tail pinch %Analgesia		Hot plate median latency s.	
		30 min.	90 min.	30 min.	90 min.
saline	5	20	0	7.5	7
mepyramine maleate(10)	5	10	0	7	8.5
mepyramine maleate(20)	5	60	20	10	10
saline	10	70	10	12	8.5
mepyramine maleate(10)	10	80	0	10	5
mepyramine maleate(20)	10	90	70	9	11
methyl cellulose	5	30	20	6.5	7
astemizole(1)	5	10	10	4.5	5.5
astemizole(10)	5	10	0	6	5
methyl cellulose	10	40	20	7	5.5
astemizole(1)	10	30	20	7	7.5
astemizole(10)	10	10	30	8	6

These results suggest that the site of interaction of H1 antagonists with opioids is central rather than peripheral.

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MODIFICATION OF THE APPETITIVE EFFECTS OF PHYSOSTIGMINE BY THE OPIOID ANTAGONIST NALTREXONE

Helen C. Jackson* & R.D.E. Sewell, Division of Pharmacology, Welsh School of Pharmacy, UWCC, P.O. Box 13, Cardiff CF1 3XF *Present address: Reckitt & Colman Psychopharmacology Unit, The Medical School, University Walk, Bristol BS8 1TD.

There is now ample evidence implicating both cholinergic agents and endogenous opioids in the control of food and water consumption (Defeudis, 1974; Morley et al., 1983; Fuller, 1984). However, the possibility of a connection between the stimulatory appetitive effects of these compounds has not previously been investigated. Thus, in the current study, we have examined food and water intake in freely-feeding rats following administration of the acetylcholinesterase inhibitor physostigmine, either alone or in combination with the opioid antagonist naltrexone.

Individually-housed male Wistar rats (300-350g) were allowed free access to powdered standard rat diet and tap water at all times. Physostigmine (B.D.H.) and naltrexone (Endo) were injected i.p. in a dose volume of 1 ml/kg. Physostigmine was dissolved in 1% tartrate and naltrexone in 0.9% saline. Experiments were carried out during the daylight period and animals were given 30 min pretreatment with the acetylcholinesterase inhibitor in the interaction studies. Feeding jars and water bottles were weighed at the time of drug administration and after 1,2 and 4h to enable calculation of mean group cumulative intakes g/kg rat weight $\pm$ s.e. mean (n=6). Statistical comparisons were made using the analysis of variance and the Dunnett's test.

Physostigmine (1 mg/kg) produced a significant increase (311%, $P<0.05$) in food intake which became evident 2h after drug administration. Cumulative food intakes of drug-treated animals were still greater than those of controls at the 4h reading (261%, $P<0.05$). Lower doses of physostigmine (0.01, 0.1 mg/kg) had no significant effects on feeding behaviour. Water consumption was also increased by physostigmine (132%, 0.1 mg/kg; 96%, 1 mg/kg; $P<0.05$) although this response did not emerge until the second half of the 4h trial. Rats treated with the two highest doses of physostigmine displayed overt signs of cholinergic stimulation - including tremor, lachrymation, salivation and teeth-chattering. The hyperphagia and hyperdipsia induced by physostigmine (1 mg/kg) was significantly attenuated by naltrexone (1 mg/kg i.p., $P<0.01$). This low dose of the opioid antagonist reverses the appetitive effects of a variety of opioid agonists whilst having no effects on day-time feeding and drinking levels per se (Jackson & Sewell, 1986).

The present findings suggest that the stimulatory effects of cholinergic agents on food and water intake may be mediated, at least in part, by the release of endogenous opioids, and in accordance with this hypothesis, both physostigmine and the cholinesterase inhibitor di-isopropylphosphofluoridate have been shown to produce naloxone-reversible antinociception in rats and mice (Harris et al., 1969; Koehn et al., 1980).

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IDENTIFICATION OF A SIGMA SITE IN GUINEA-PIG ADRENAL GLAND

R.H. Blades, A.K. Knight and E.H.F. Wong. Merck Sharp and Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex, CM20 2QR.

Sigma recognition sites are non-opioid sites with affinity for benzomorphan opiates such as η-allylnormetazocine (SKF 10,047) and were shown to be present in the brain (Sonders et al., 1988). Recent reports of sigma binding in the periphery suggests high densities of sigma sites in the liver (Samovilova et al., 1988) and endocrine tissues (Wolfe et al., 1988). We have investigated sigma binding in the periphery by studying the binding properties of 1,3-Di(2-[5-^{3}H]tolyl) guanidine ([^{3}H]-DTG; Weber et al., 1986) in the guinea pig adrenal gland.

Binding studies were carried out in homogenates of whole adrenal gland obtained from male Duncan-Hartley guinea pigs (250-300g). Tissue was homogenised in 40 volumes of 50mM Tris HCl, 1mM EDTA and centrifuged at 48,000g for 20 min. The pellet was washed in 40 volumes of the above buffer and centrifuged at 48,000g for 20 min. The pellet was resuspended in 50mM Tris(pH 7.4) to the required volume. 2.5nM [^{3}H]-DTG was used to label the sigma site, non-specific binding was defined by 10 μM haloperidol.

Binding of [^{3}H]-DTG to the adrenal site was saturable and reversible, with affinity (Kd) and site density (Bmax) values (mean ± S.E.M., n = 3) of 25.6 ± 1.1nM and 4.83 ± 0.57 pmol/mg protein respectively. The pharmacological specificty of the adrenal site was investigated by displacement of [^{3}H]-DTG binding by prototypic sigma ligands. The adrenal site showed a close correlation to the cerebellar sigma site in guinea pig brain (crude P_2, Weber et al., 1986), in terms of rank order and potency (Table 1). Preliminary autoradiographic studies indicate a concentration of these sites in the adrenal cortex.

Table 1. Displacement of [^{3}H]-DTG (2.5nM) Binding in Guinea Pig Adrenal and Cerebellar Membranes

	a) Adrenal		b) Cerebellum	
	Kapp (nM)	nH	Kapp (nM)	nH
Haloperidol	14.9 ± 4.5	1.18 ± 0.09	5.2 ± 1.3	0.85 ± 0.07
DTG	44.4 ± 4.8	1.02 ± 0.04	40.7 ± 8.1	0.88 ± 0.02
(±) BMY14802	92.7 ± 8.2	1.00 ± 0.09	191.9 ± 64.9	0.69 ± 0.05
(±) Pentazocine	242.4 ± 4.4	0.87 ± 0.07	69.1 ± 10.9	0.63 ± 0.03
(+) SKF 10,047	7256.0 ± 562.5	0.53 ± 0.07	1219.3 ± 282.0	0.45 ± 0.006

Values were expressed as mean ± S.E.M., n = 3; nH = Hill coefficient.

We have shown the presence of a [^{3}H]-DTG binding site in guinea pig adrenal cortex which has identical pharmacological specificity to that of the sigma site in the brain. The identification of sigma sites in this tissue might facilitate our understanding of the pharmacological relevance of these sites.

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BARBITURATES BUT NOT STEROIDS MODULATE THE GABA AUTORECEPTOR

C. Ennis and M.C.W. Minchin, Wyeth Research, Huntercombe Lane South, Maidenhead, Berkshire. SL6 OPH. U.K.

The GABA-A receptor is known to exist in a complex with a benzodiazepine receptor and a chloride ion channel linked to a barbiturate binding site. We have reported previously that the GABA autoreceptor which modulates the release of [^{3}H]-GABA from slices of rat cerebral cortex appears to be coupled to a benzodiazepine receptor (Ennis & Minchin, 1988). The aim of the present study was to determine if the GABA autoreceptor was also modulated by compounds thought to interact with the chloride ion channel component of the GABA-A receptor complex.

Slices of rat cerebral cortex (0.25 x 0.25 x 2mm) were preloaded with [^{3}H]-GABA (0.1 µM, specific activity 59 Ci.mmol^{-1}) and superfused at 37°C with Krebs-Henseleit solution containing amino-oxyacetic acid (10 µM) at a rate of 0.4 ml min^{-1}. Fractions of the superfusate were collected every 4 min. Two 4 min pulses of Krebs solution containing 25mM K^+ were administered 68 (S_1) and 92 (S_2) min after the start of the superfusion. Drugs were added to the superfusing medium immediately after S_1. The results were calculated as the S_2/S_1 ratio and statistical analysis was performed using the Mann-Whitney U test, 2 tailed.

Muscimol, as reported previously, produced a concentration-dependent inhibition of K^+-evoked [^{3}H]-GABA release with a pD_2 value of 7.73 ± 0.07 (n = 12). The inhibition produced by muscimol at 1.0 µM was 40 ± 3%; higher concentrations produced an increase in the basal release of tritium. The effect of muscimol (0.1 µM) was reversed by the antagonist picrotoxin with an IC_{50} of 0.15 ± 0.03 µM. In the presence of (±)dimethylbarbituric acid ((±)DMBB) (100 µM) or pentobarbitone (100 µM), there was a significant shift to the left in concentration-effect curve to muscimol. The pD_2 value for muscimol in the presence of (±)DMBB (100 µM) was 9.89 ± 0.34 and that in the presence of pentobarbitone (10^{-4} M) was 8.75 ± 0.28. The maximum inhibition produced by muscimol (1.0 µM) was also increased significantly to 61.8 ± 7% and 50 ± 4% respectively. Neither compound significantly affected the K^+-evoked release of [^{3}H]-GABA in the absence of muscimol.

It has been reported also that steroids in the pregnane series can potentiate responses of the GABA-A receptor (Harrison & Simmonds, 1984; Peters et al, 1988) possibly by a mechanism similar to that of the barbiturates. However in the presence of the steroid anaesthetic alphaxalone (1.0 µM) there was no change in either the pD_2 value for muscimol (7.65 ± 0.29) or the maximum inhibition produced. Pregnanolone (0.1 and 1.0 µM) produced a significant and concentration-related increase in the K^+-evoked release of [^{3}H]-GABA in the absence of muscimol but at lower concentrations (0.01 µM) there was no effect on the response to muscimol (0.1 µM).

Thus the results suggest that the GABA autoreceptor which inhibits the release of [^{3}H]-GABA from slices of rat cerebral cortex is modulated by barbiturates but does not appear to be modulated by steroids. This supports the recent suggestion that these two classes of compound may not act at the same site (Peters et al., 1988).

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SR95103 ACTS AS A GABA ANTAGONIST IN ASCARIS SUUM MUSCLE

By A.H. Duittoz & R.J. Martin. Department of Preclinical Veterinary Sciences, R.(D.)S.V.S., University of Edinburgh, Summerhall, Edinburgh EH9 1QH.

The distinctive properties of the GABA receptor in the parasite nematode Ascaris suum are of interest since they may be exploited therapeutically (Martin, 1987). It has been reported that the classical $GABA_a$ antagonists and the specific $GABA_b$ agonist Baclofen have no effect on GABA induced hyperpolarisations (Holden-Dye et al., 1988). We have examined effects of Picrotoxin, Bicuculline and the arylaminopyridazine derivatives; SR95531 and SR95103 (10^{-3}M), on cumulative GABA conductance dose-response relationships using techniques described previously (Martin, 1980). Only SR95103 displayed an antagonist effect (Fig. 1). Dose-response curves to GABA were shifted to the right in a parallel manner (10^{-4}M), (Fig. 2). A modified Schild analysis (Williams et al., 1988) suggested that SR95013 (tested at 10^{-4}, $3x10^{-4}$, 10^{-3}M) was a competitive antagonist and that two molecules of GABA but only one of SR95103 interacted with the receptor.

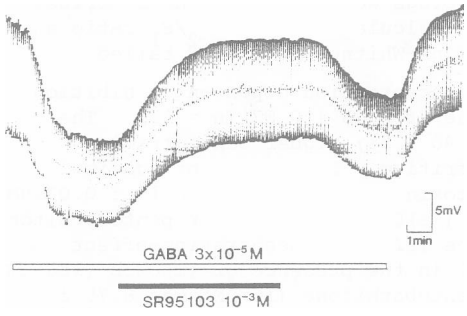

Fig. 1
Effect of GABA ($3x10^{-5}$M) and GABA ($3x10^{-5}$M) + SR95103 (10^{-3}M) on membrane input conductance. Glass microelectrodes: 10-20MΩ filled with K acetate 2M. Current pulse: 40nA, 2s, hyperpolarising. Ringer solution (mM): NaCl 134, KCl 3, $MgCl_2$ 15.7, Glucose 3, TRIS 5, adjusted to pH 7.6 with maleic acid. Resting membrane conductance: g=2.7μS; GABA ($3x10^{-5}$M) induced conductance change g=1.1μS; GABA ($3x10^{-5}$M) + SR95103 (10^{-3}M) g=0.3μS; GABA recovered its effect on conductance when SR95103 was washed.

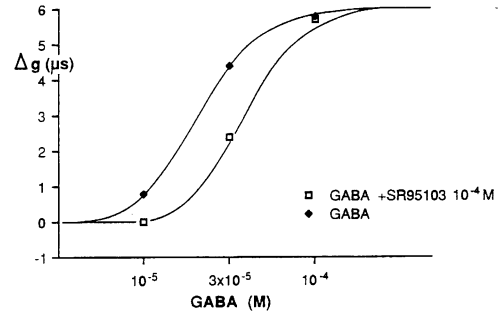

Fig. 2
Dose-response curves to GABA and GABA + SR95103 (10^{-4}M). Dose-response curves were fitted to the modified Hill equation by non linear regression.

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A STUDY OF TOLERANCE TO Ro 17-1812, A PARTIAL AGONIST BENZODIAZEPINE, AND THE CROSS-TOLERANCE PRODUCED BY DIAZEPAM

P. Boyland, J.P. Gent and M. Feely. Dept. of Pharmacology, University of Leeds, LS2 9JT

There are substantial differences between benzodiazepines (BDZs) in respect of both rate and degree of anticonvulsant tolerance development (Haigh & Feely, 1988a). Cross-tolerance between BDZs has been described in both animals (Gent et al, 1986) and man (Crawford et al, 1987). Recently it has been shown that the partial agonist benzodiazepine Ro 16-6028 exhibits little if any anticonvulsant tolerance in mice (Haigh & Feely, 1988b). Here, we describe a study of tolerance with another partial agonist, Ro 17-1812, and the cross-tolerance produced by diazepam.

Anticonvulsant activity was assessed in mice (adult, male, Tuck No. 1, 25-40g, groups of 5) using a slow intravenous infusion of pentylenetetrazol (PTZ). Diazepam and Ro 17-1812 were dissolved in a propylene glycol-based vehicle and given s.c. 45 mins and 15 mins respectively before testing with PTZ (see Gent et al, 1985). The minimum convulsant dose (MCD) required to elicit a clonic convulsion in each animal was measured. The studies of tolerance were carried out in animals dosed with diazepam or Ro 17-1812 twice daily, i.p., for 16 days. The cross-tolerance study was performed in mice dosed i.p. twice daily for 48 hours with diazepam and then given Ro 17-1812.

MCD (mg kg^{-1} of PTZ) following repeated 12 hourly administration of (a) diazepam, (b) Ro 17-1812 in "naive" animals and (c) Ro 17-1812 in 'diazepam tolerant' animals: Results expressed as mean (s.e. mean)

Period of dosing	**Acute**	**24 hours**	**48 hours**	**96 hours**	**8 days**	**16 days**
a) Diazepam (1.5 mg kg^{-1})	98.64 (1.84)	- -	81.64 (4.34)	64.27 (3.43)	67.15 (1.92)	73.76 (1.97)
b) Ro 17-1812 (1 mg kg-1)	116.10 (2.57)	115.79 (4.91)	113.75 (3.64)	113.21 (5.38)	112.22 (3.81)	107.25 (1.85)
c) Ro 17-1812 (1 mg kg-1) after diazepam	87.36 (3.16)	99.84 (2.99)	99.31 (2.82)	111.59 (1.98)	87.79 (5.97)	101.43 (2.29)

In summary, no significant tolerance developed with Ro 17-1812 ($p > 0.05$, single classification analysis of variance), extensive tolerance developed rapidly in the case of diazepam and diazepam produced cross-tolerance to Ro 17-1812. After switching from diazepam to Ro 17-1812 there was an overall trend towards recovery of anticonvulsant activity.

We believe that the efficacy of one BDZ as an anticonvulsant may be reduced if a patient takes (or has recently taken) a BDZ with greater tolerance-inducing potential. The results of the present animal study, when considered with the observation of cross-tolerance between BDZs during the treatment of status epilepticus (Crawford et al, 1987), demonstrate the need for further investigations in this area.

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CONCURRENT EVALUATION OF BENZODIAZEPINE TOLERANCE AND DEPENDENCE USING AN INTRAVENOUS PENTYLENETETRAZOL TEST

M. Feely, S. Peaker, J.P. Gent and P. Boyland. Departments of Medicine and Pharmacology, University of Leeds, LS2 9JT

The use of pentylenetetrazol (PTZ) infusion as a convulsive 'challenge' has proved particularly useful for studying benzodiazepine (BDZ) tolerance (Haigh & Feely, 1988). BDZ tolerance and dependence appear to be closely related and may arise from the same adaptive mechanisms (Lader & File, 1987). Withdrawal seizures constitute one of the more dramatic and earliest recognised manifestations of BDZ dependence in man. We decided to see if we could also use the i.v. PTZ challenge, with an established protocol for evaluating tolerance, to study the proconvulsant effect of BDZ withdrawal, and to compare lorazepam with clonazepam. Clonazepam appears to have less tolerance-inducing potential than most conventional BDZs (Haigh & Feely, 1988), lorazepam appears to be particularly associated with dependence in man and both drugs have a fairly similar duration of action in the mouse.

Doses and times for testing were chosen as a result of preliminary experiments which determined acute (single dose) time profiles and log-dose response curves. To induce tolerance both BDZs were injected i.p. twice daily for 3 days. To test anticonvulsant activity clonazepam and lorazepam were given s.c. 30 min and 1h before challenge with PTZ (see Gent et al., 1985). To assess dependence PTZ challenge was performed 10, 12, 14 & 16 hours after the last dose of lorazepam or clonazepam. Groups of 10 mice (adult, male, Tuck No 1, 25-40g) were used throughout; results are expressed as mean (± s.e. mean) minimum convulsant dose (MCD) of PTZ, $mg.kg^{-1}$.

Results:

		TOLERANCE		WITHDRAWAL				CONTROLS
		1st dose	3d	10h	12h	14h	16h	(vehicle only)
LORAZEPAM ($0.5\ mg\ kg^{-1}$)	mean	106.7	85.3	42.2	37.6	36.7	42.6	41.5
	s.e.m.	2.6	1.2	1.4	1.5	0.8	1.0	0.8
CLONAZEPAM ($0.25\ mg\ kg^{-1}$)	mean	113.4	98.5	41.2	41.2	42.4	41.4	43.3
	s.e.m.	1.9	3.1	0.9	1.2	1.4	1.2	0.6

Significant tolerance ($p < 0.001$, Student's t-test) was seen with both BDZs but the loss of anticonvulsant activity was less with clonazepam. No significant proconvulsant withdrawal effect was seen with clonazepam but the MCD of PTZ fell significantly below control values 12h ($p < 0.05$) and 14h ($p < 0.001$) after the last dose of lorazepam. Assays of mouse plasma using HPLC showed low levels of lorazepam (5-15 ng/ml) in many mice at 12h and 14h, whereas clonazepam was absent from 12h onwards.

The evidence of a rapid induction of lorazepam dependence is in keeping with the recent observations of Nutt & Costello (1988), who studied sensitisation to β-CCM or the inverse agonist FG-7142 following lorazepam withdrawal. The model used for the present study may be particularly useful for examining the relationship between tolerance and dependence.

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NITRENDIPINE DECREASES SEIZURE INCIDENCE DURING BENZODIAZEPINE WITHDRAWAL BUT NOT SEIZURES CAUSED BY BICUCULLINE

T. Patch[1], H. J. Little[2], R. J. Siarey[2] & S. J. Dolin[1]
[1]Clinical Pharmacology Unit, Addenbrookes' Hospital, Hills Road, Cambridge, CB2 22Q and [2]Department of Pharmacology, The Medical School, University Walk, Bristol, BS8 1TD.

Calcium antagonists are effective in decreasing or preventing seizures following withdrawal from chronic treatment with ethanol (Little et al, 1986; Littleton & Little, 1987), barbiturates (Brown et al, 1988) or N_2O (Dolin & Little, 1987). They also decrease the opiate withdrawal syndrome (Bogianni et al, 1986). We now report the effects of the dihydropyridine, nitrendipine, on seizures precipitated after chronic benzodiazepine treatment by the partial inverse agonist FG7142.

Male mice, TO strain (25-30g) were given seven daily i.p. injections of flurazepam, 40 mg/kg; controls received saline. 24h after the last injection, groups received nitrendipine, 10, 50 or 100 mg/kg, or the vehicle Tween 80 (0.5% in distilled water). 30 min later all animals were given FG7142, 40 mg/kg i.p., and were watched for 40 min by an observer who did not know the prior drug treatment.

Nitrendipine, at 50 and 100 mg/kg, significantly decreased the number of seizures following administration of FG7142 in animals which had received chronic flurazepam treatment. No seizures were seen when FG7142 was administered after treatment with nitrendipine alone or with saline.

Chronic treatment	Acute (mg/kg)	Seizure incidence
1) Flurazepam	Tween	11/20
Flurazepam	Nitr. (10)	8/19
Flurazepam	Nitr. (50)	3/20*
Flurazepam	Nitr. (100)	4/19*
2) Flurazepam	Tween	7/10
Flurazepam	Nitr. (50)	2/10*

* $P < 0.05$, Fisher's Exact test. All animals were given FG7142, 40 mg/kg

This effect of nitrendipine was compared with its action against the GABA antagonist bicuculline, in naive mice (TO strain). At 50 and 100 mg/kg i.p. nitrendipine did not significantly alter the convulsions due to bicuculline (bic), given by tail vein infusion (Nutt et al, 1980) or i.p., at 6 mg/kg. Nitrendipine (nitr.) was injected i.p. 30 min before the i.v. infusions and 10 min before the i.p. bicuculline.

	Tween vehicle	Nitr. 50 mg/kg	Nitr. 100 mg/kg
I.v. thresholds, mg/kg bic.(n):	0.44±0.06 (6)	0.55±0.02 (7)	0.6±0.06 (7)
Conv. incidence, i.p. bic.	7/8	7/8	7/8

$P>0.1$ in all comparisons with controls (t-test for i.v. bic., Fisher's Exact test for i.p. bic).

Nitrendipine appeared therefore to show selectivity for seizures produced by the inverse agonist during benzodiazepine withdrawal. This is in agreement with the selectivity shown previously for drug withdrawal seizures.

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LITHIUM ENHANCES THE INHIBITORY EFFECTS OF FLUOROALUMINATE ON INOSITOL PHOSPHOLIPID HYDROLYSIS IN RAT BRAIN

P. Whitworth and D.A. Kendall, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Lithium (Li^+) is the most effective therapy in the treatment of manic-depression but its mechanism of action remains unknown. Reports that Li^+ blocks GTP binding in cerebral cortical membranes suggest that G proteins may be a target for its action. Fluoroaluminate ions (AlF_4^-) have been shown to stimulate adenylate cyclase via G proteins and to stimulate or inhibit inositol phospholipid hydrolysis in intact cells, possibly via the activation of stimulatory and inhibitory G proteins (Godfrey & Watson, 1988). We have examined the effect of Li^+ on the inhibition of inositol phospholipid hydrolysis due to AlF_4^- in rat brain slices.

Inositol phosphate (InsP) accumulation was measured in slices of rat cererbal cortex labelled with ^{3}H-inositol. Total InsP or individual inositol polyphosphates were separated by anion exchange chromatography or HPLC respectively (Whitworth & Kendall, 1988). Additions of Li^+ (5 mM) were made 5 min before AlF_4^-. AlF_4^- (NaF 20 mM/AlF_4^- 10^{-5} M) was added 5 min before the addition of the agonist or depolarising agent.

AlF_4^- alone stimulated inositol phospholipid hydrolysis (1633±129 d.p.m. above basal) and this response was potentiated by Li^+ (2736±247 d.p.m.). Addition of AlF_4^- produced a 43% reduction in the accumulation of total InsP due to the muscarinic agonist carbachol (1 mM, 45 min stimulation) and this was potentiated by Li^+ (73% reduction). The reductions were in the maximal response to carbachol with no change in the EC_{50}. Responses due to noradrenaline (100 µM) plus AlF_4^- and KCl (50 mM) plus AlF_4^- were less than additive with respect to the responses to either agonist or AlF_4^- alone. The addition of Li^+ enhanced this apparent reduction such that the combinations of noradrenaline plus AlF_4^- and KCl plus AlF_4^- produced responses which were only 41% of the responses that would have been expected if the effects of the stimulating agent and AlF_4^- were strictly additive. The response to histamine (1 mM) was not inhibited by AlF_4^- in the presence or absence of Li^+ and the inhibition of the response to A23187 (3×10^{-5} M) by AlF_4^- (43%) was unaffected by LiCl.

$InsP_1$ accumulation due to 5 min stimulation with a combination of carbachol and AlF_4^- was additive with respect to either agent alone (147000 dp.m. over basal). However in the presence of Li^+ this response was reduced to 52500 d.p.m. (64% reduction). Both Li^+ and AlF_4^- reduced Ins 1,4,5P_3 accumulation due to carbachol (31% and 67% respectively), and caused a greater reduction when combined (91%). $InsP_4$ accumulation due to carbachol was reduced by AlF_4^- alone (84% reduction) but not by Li^+. However, when used in combination Li^+ potentiated the inhibition due to AlF_4^- (94%).

These findings provide indirect evidence that Li^+ might potentiate the effects of inhibitory G proteins linked to inositol phospholipid hydrolysis.

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EFFECT OF SURGICAL AND PHARMACOLOGICAL ADRENALECTOMY ON NORADRENALINE-STIMULATED SECOND MESSENGER RESPONSES IN RAT BRAIN

S. Bharmal, D.A. Kendall, J.P. Robinson, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

In rat cerebral cortex slices, potentiation of β-adrenoceptor stimulated cyclic AMP formation and inositol phospholipid hydrolysis both appear to be mediated through α adrenoceptors. Cyclic AMP formation due to noradrenaline is increased in adrenalectomised (ADX) rats (Mobley and Sulser, 1980) and in rats treated with the steroid synthesis inhibitor, metyrapone (Roberts et al. 1984). In this study, we have compared the effects of surgical and pharmacological adrenalectomy on cyclic AMP and inositol phosphate responses to noradrenaline in rat cerebral cortex slices.

Male Sprague-Dawley rats (300g) were injected with metyrapone (MET, 2-methyl -1,2-di-3-pyridyl-1-propanone; 50mg/kg i.p.) or vehicle 2hr prior to sacrifice. For adrenalectomy studies, rats were anaesthetised with Brietal and both adrenal glands removed. Rats were allowed to recover and were given free access to 0.9% saline to maintain salt balance for 21-24 days before sacrifice. Sham operated animals served as controls. Cyclic AMP formation in cerebral cortex slices in response to isoprenaline (ISO) and noradrenaline (NA) was measured by the ^{3}H-adenine prelabelling method of Shimizu et al. (1969). NA stimulated inositol phospholipid breakdown was monitored by measuring total ^{3}H-inositol phosphate accumulation (Kendall et al., 1985).

Cyclic AMP formation in response to NA stimulation typically produced a 5 fold increase over basal in brain slices from control and sham operated animals. In brain slices from MET-treated animals, the maximal response to NA was significantly greater than vehicle injected controls ($P<0.01$, see table). This increase was prevented by treating rats with corticosterone (5mg s.c.) or dexamethasone (1mg s.c.). In adrenalectomised rats the increase in NA stimulated cyclic AMP was more pronounced. In both cases, the increase was due to a change in the α-adrenoceptor mediated portion of the response, since there were no differences in the responses to isoprenaline. Noradrenaline stimulated inositol phospholipid hydrolysis was not significantly increased by adrenalectomy or by MET-treatment (see table).

	cyclic AMP response (n)	inositol phosphate response (n)
CONTROL	100 ± 5 (6)	100 ± 3 (4)
MET	143 ± 10 (6)*	105 ± 6 (4)
SHAM	100 ± 6 (3)	100 ± 4 (3)
ADX	164 ± 10 (3)*	129 ± 13 (6)*

* $P<0.05$ Results are expressed as a % of control response to 100 μM NA.

It seems therefore that rapid changes in the α-adrenoceptor potentiation of cyclic-AMP formation can be provoked by inhibition of corticosteroid synthesis, but that the α_1-adrenoceptor mediating inositol phospholipid hydrolysis is not under the control of adrenal steroids.

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THE POTENCIES OF SEVERAL INJECTABLE ANAESTHETICS ON A SPINAL, NOCICEPTIVE REFLEX IN BOTH DECEREBRATE AND ANAESTHETISED RATS

N.A. Hartell* and P.M. Headley, Department of Physiology, University of Bristol The School of Medical Sciences, University Walk, Bristol BS8 1TD, U.K.

Many electrophysiological studies of spinal sensory processing in vivo have been undertaken in the presence of a wide variety of anaesthetics. To what extent such compounds influence the results obtained is not fully understood. The effects of injectable anaesthetics of four widely used classes were compared on the response to controlled noxious pinch stimuli of single motor units of flexor muscles in the hind limb of the adult spinalised rat.

Sixty one rats were prepared under halothane anaesthesia. Tracheal, arterial and venous cannulae were inserted and a small laminectomy made in the low thoracic region so that the spinal cord could be cut. Wound surfaces were kept to a minimum and were covered with lignocaine. One group of rats was then decerebrated (n=18); in the others anaesthesia was maintained either with alpha-chloralose (n=20) or with alphaxalone/alphadolone (Saffan; Glaxo) (n=23). Recordings were made from flexor muscle motor units via microwires inserted through percutaneous needle electrodes. Noxious pinch stimuli were cycled every three minutes and responses quantified as the number of spikes evoked per stimulus. Dose ($\log_2$) response curves were constructed for each drug tested. Data were pooled and were compared between the three groups of rats.

The dissociative anaesthetic ketamine (0.5-16 mg/kg i.v.), and the short-acting barbiturate methohexitone (1-8 mg/kg i.v.), showed no significant difference in potency between the three different experimental groups. Similarly, the mu opioid fentanyl did not display any difference in potency (1-8 ug/kg i.v.). In contrast, Saffan (0.5-8 mg/kg i.v.) was 8-16 fold weaker in those animals maintained under Saffan anaesthesia; similarly, alpha-chloralose (20-80 mg/kg i.v.) proved to be between two and four times less potent under chloralose anaesthesia. These differences could be due to no more than the effects of the test doses being superimposed on different parts of the dose response curve in the different experimental groups. Alternatively they could result from different levels of neuronal excitability between the experimental groups, since any such variation can result in apparent differences in the potency of test agents (e.g. Headley & Parsons, 1989). The latter proved unlikely since the mean firing rate of the motorneurones during pre-Saffan control periods was not significantly different (Mann-Whitney U-test) between decerebrate (29.6 $\pm$ 2.8 spikes/sec; mean $\pm$ s.e.m., n=10), Saffan (22.2 $\pm$ 2.1, n=11) and chloralose (24.6 $\pm$ 2.8, n=13).

The similar effectiveness between decerebratre and anaesthetized states, of drugs not related to the basal anaesthetic, lends credence to the results of such pharmacological tests performed on anaesthetised animals.

N.A.H. is an M.R.C. scholar.

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THE EFFECT OF TWO INTRAVENOUS ANAESTHETICS ON SEIZURES PRODUCED BY 4-AMINOPYRIDINE IN THE RAT

Wardley-Smith, Bridget and Wann, K.T. HPNS Group, Clinical Research Centre, Watford Road, Harrow, HA1 3UJ.

The effectiveness of the anaesthetics alphaxalone-alphadalone (Althesin) and methohexitone has already been studied against the convulsant actions of bicuculline, strychnine and high pressure. Both anaesthetics protect against bicuculline and strychnine seizures; in contrast Althesin protects against hyperbaric seizures, but methohexitone potentiates them (Wardley-Smith, Little & Halsey, 1988). We have now tested the interaction of these anaesthetics with seizures produced by 4-aminopyridine (4-AP), a drug which like high pressure is convulsant (von Haxthausen 1955), modifies anaesthetic effects (Agoston et al., 1980) and blocks the K^+ 'A' channel (Segal et al., 1984).

Male Sprague-Dawley rats (250-400g) were used in all experiments. Althesin or methohexitone was infused intravenously via a motor driven syringe connected to a needle in the tail vein. Once anaesthesia was stable (approx 5 min, Althesin 0.66 mg/kg/min n = 5, methohexitone 1.10 mg/kg/min, n = 5) 4-AP was infused i.v. simultaneously with the anaesthetic. The times to whole body jerks (WBJ) and clonic seizures (CS) were recorded; in addition the time to tremor was noted. Of necessity, control experiments involved rats which were not anaesthetized. Althesin controls were carried out by infusing cremophor in saline for 5 min followed by simultaneous infusion of 4-AP. Methohexitone controls were similar, except that phosphate buffered saline (PBS) adjusted to the same pH as methohexitone (pH 11) was infused.

Results are shown in the table below and were compared using Students 2-tailed t-test.

Mean dose (mg/kg) of 4-AP (s.e.m.) to produce whole body jerks and clonic seizures

	WBJ	CS
Althesin	10.6 (0.4)	12.7 (0.4)
Cremophor/saline	7.0 (0.7)	9.4 (0.7)
	p = 0.002	p = 0.003
Methohexitone	5.6 (0.4)	8.6 (0.4)
PBS pH 11	8.5 (0.5)	10.6 (0.3)
	p = 0.02	p = 0.002

In addition, Althesin increased the amount of 4-AP required to produce tremor, although methohexitone had no effect.

These results show that Althesin protects against whereas methohexitone potentiates motor events produced by 4-AP. In this respect the action of 4-AP resembles that of high pressure, unlike other convulsants tested (Wardley-Smith, Little & Halsey, 1988). One possibility is that blockade of K+ channels is responsible for hyperbaric myoclonic jerks and seizures.

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CHARACTERISATION OF P_2-PURINOCEPTORS IN HUMAN SMALL PULMONARY ARTERIES

S F Liu, D G McCormack, T W Evans, P J Barnes, Department of Thoracic Medicine, National Heart and Lung Institute, London, SW3 6LY, U.K.

The distribution of P_2-purinoceptors varies between species and different vascular beds (Kennedy and Burnstock, 1985; Houston et al, 1987; Ralevic and Burnstock, 1988). ATP (a P_2-purinoceptor agonist) relaxes human pulmonary arteries (Greenberg et al, 1987), however the characteristics and distribution of P_2-purinoceptors in human pulmonary arteries have not been classified. P_{2X} and P_{2Y} subtypes of P_2-receptors are defined according to agonist potency series of the adenosine triphosphate (ATP) analogues, α,β-methylene-ATP (α,β-meATP), β,γ-methylene-ATP (β,γ-meATP), 2-methylthio-ATP (2m.S.ATP) and ATP. The P_{2x}-receptor is unique in that it can be selectively desensitized by α,β-meATP. We attempted to characterise P_2-purinoceptors in isolated human pulmonary arteries. Fresh human tissue was obtained from patients undergoing thoracotomy. Small pulmonary arteries were dissected, cut into rings 4-5 mm in length and mounted over a pair of rigid wires connected to a force transducer and suspended in an organ bath containing Krebs-Henseleit solution (37° C). In some of the experiments, the vascular endothelium was removed by rotating a small probe within the lumen and was confirmed by a lack of relaxant response to acetylcholine (0.1-10 uM). To study relaxation, the rings were precontracted with 1 uM prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and cumulative dose-response curves for the ATP analogues obtained. To study contraction, agonists were added to the organ bath when the vessels were at resting tension and cumulative dose-response curves obtained. Contraction was calculated as a percentage of a maximal KCl contraction (60 mM). Relaxation was expressed as a percentage of the maximal contraction to 1 uM $PGF_{2\alpha}$. The rank order of potency in causing relaxation was 2m.S.ATP >> ATP >> β,γ-meATP = α,β-meATP, demonstrating this was mediated through a P_{2y}-receptor (Burnstock and Kennedy, 1985). Removal of the endothelium had no significant effect on the relaxant response to ATP analogues. The rank order of potency of the analogues for inducing contractile responses was α,β-meATP, =β,γ-meATP > ATP > 2m.S.ATP, consistent with this contraction acting through P_{2x}-receptor. After vessels were treated with 100 uM α,β-meATP (to desensitise P_{2x}-receptor), the contratile response to β,γ-meATP was abolished. Removal of the endothelium had no significant effect on the contractile response to ATP analogues.

We conclude that both vasoconstrictor, P_{2x}-, and vasodilator, P_{2y}-receptors are present in human small pulmonary arteries and probably reside on smooth muscle. P_{2y}-receptors may also be located on the endothelium. P_2-purinoreceptors may be important in the regulation of pulmonary blood flow in human.

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CO-EXISTENCE OF A1 AND A2 ADENOSINE RECEPTORS IN THE GUINEA-PIG AORTA

S.M. Stoggall and J.S. Shaw, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire SK10 4TG.

On the basis of their inhibition and stimulation of adenylate cyclase and their differing affinities for adenosine and its analogues (Londos and Wolff, 1977; Collis and Brown, 1983) adenosine receptors have been subdivided into the A_1 and A_2 types. The aim of this study was to characterise the adenosine receptor subtype responsible for changes in vascular tone in guinea-pig isolated aortic rings.

Aortic rings from male Dunkin Hartley guinea-pigs were suspended in Krebs solution at 37°C, gassed with 5% CO_2 in O_2 under a resting tension of 1g . The adenosine uptake inhibitor, dipyridamole (5μM), was present throughout . Changes in tone were measured isometrically. The tissues were preconstricted with phenylephrine at a previously determined EC_{50} concentration (in the range 0.7 to 2μM). Cumulative dose response curves to adenosine, 5'-(N-ethyl)carboxamido-adenosine (NECA), 2-chloroadenosine (2CA), N^6(R-2-phenylisopropyl)-adenosine (R-PIA) and N^6-cyclohexyladenosine (CHA) were constructed. In all cases, $n \geq 8$.

The main response to adenosine and NECA was relaxation. R-PIA, CHA and 2CA all produced biphasic dose response curves. Between 0.1μM and 1μM of each agonist a concentration dependent contraction occurred. This was followed at higher concentrations by relaxation. R-PIA elicited the largest contraction whilst 2CA and CHA produced somewhat smaller responses.

TABLE 1		RELAXATION EC_{50} μM	MAXIMUM CONTRACTION (% PHENYLEPHRINE RESPONSE)
Potencies of adenosine analogues for inducing contraction and relaxation of the guinea-pig aorta.	NECA	0.34 ± 0.03	0
	ADENOSINE	2.9 ± 0.25	0
	2CA	5.9 ± 1.1	11.3 ± 3.8
	R-PIA	11.3 ± 2.1	18.3 ± 3.1
	CHA	>10	11.3 ± 3.5

The rank order of potency of adenosine and its analogues for inducing aortic relaxation is in keeping with that of the A_2 subtype (Collis and Brown, 1983; Edvinsson and Fredholm 1983). However the analogues R-PIA, CHA, and 2CA were found to contract the aorta at concentrations lower than those required for relaxation. The order of potency for this effect is indicative of an A_1 type response. The A_1 selective antagonist PD 116948 (8-cyclopentyl-3,7-dihydro-1,3-dipropyl-1H-purin-2,6-dione) (0.5μM) abolished the contraction produced by R-PIA. This concentration had no effect on the relaxant response of the aorta to adenosine, but has previously been shown to antagonise the A_1 mediated reduction in atrial rate by R-PIA (Haleen et al., 1987). This evidence suggests the existence of both A_1 and A_2 adenosine receptor subtypes in the guinea-pig aorta preparation.

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INTERACTIONS OF P1-ADENOSINE AND MUSCARINIC RECEPTOR AGONISTS WITH Bay K 8644 IN GUINEA-PIG LEFT ATRIA AND PAPILLARY MUSCLES

R.A. Urquhart & K.J. Broadley, Department of Pharmacology, Welsh School of Pharmacy, University of Wales College of Cardiff, PO Box 13, Cardiff CF1 3XF

In atria, agonists at muscarinic and P1-adenosine receptors produce negative inotropy at least in part by increasing K^+ conductance, which decreases action potential duration and thus decreases the time for Ca^{2+} influx. P1-receptor agonists such as N^6-(L-2-phenylisopropyl)-adenosine (PIA) or muscarinic agonists such as carbachol (CAR) should therefore be functional antagonists of positive inotropes which produce their effects by increasing the slow Ca^{2+} inward current. PIA has been reported to competitively antagonize the responses of the Ca^{2+} channel activator Bay K 8644 (Bay K) in atria but CAR was ineffective (Caparrotta et al. 1987). In the light of this surprising finding we have re-evaluated the effects of PIA and CAR upon Bay K concentration-response curves in isolated left atria (LA) and also examined their effects in papillary muscles (PM).

Isolated paced LA and PM (2Hz, 5ms, threshold voltage + 50%) were set up in Krebs-bicarbonate solution at 37.5°C, gassed with 5% CO_2 in O_2. Isometric tension was recorded. Preliminary experiments indicated that the construction of two satisfactory Bay K concentration-response curves in a single tissue was not possible even after prolonged washout. Therefore, in each preparation a single curve was constructed. In test experiments, 6-7 mins before commencing the Bay K curve, PIA (0.52μM) or CAR (0.17μM) were added, which reduced naive LA tension by approx. 50%. Tenfold greater concentrations were used in PM. Geometric mean ($n \geq 4$) EC50 values and mean maximum tension increases for Bay K are shown below. Statistical comparisons between values in absence or presence of PIA or CAR were made by Student's t-test (*$P < 0.05$).

	PIA		CAR	
	absence	presence	absence	presence
LA EC50 (nM)	320	1130*	430	590
Max.Tens.Inc.(g)	0.963	0.645*	0.74	0.74
PM EC50 (nM)	170	170	180	130
Max.Tens.Inc.(g)	0.178	0.166	0.207	0.181

Bay K produced dose-dependent positive inotropic responses in LA and PM. In LA, PIA significantly depressed the Bay K maximum and significantly increased the EC50, whereas CAR failed to significantly affect Bay K. The mechanism of the PIA antagonism is therefore unlikely to be K^+ channel-mediated since both CAR and PIA are reported to open common K^+ channels (Kurachi, 1986). Since Bay K does not increase cAMP levels (Bohm et al. 1985) this is also an unlikely site of the PIA action. Therefore, the mechanism of the PIA antagonism is uncertain, however it is clearly not a simple competitive antagonism as suggested by Caparrotta et al. (1987), since PIA significantly reduces the Bay K maximum.

In PM, neither PIA nor CAR, at 10 times the atrial concentration, significantly modified the Bay K response. This is compatible with suggestions that raised cAMP levels are necessary to reveal either P1 (Bohm et al. 1985) or muscarinic receptor (Endoh et al. 1985) effects in ventricular muscle.

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THE ACTION OF ADENOSINE RECEPTOR AGONISTS ON HUMAN ISOLATED UMBILICAL ARTERY

A. Leoutsakos, A.D. Hughes, G.N. Martin, P.S. Sever. Clinical Pharmacology, St. Mary's Hospital Medical School, London W2.1NY.

Adenosine exerts a variety of actions on the cardiovascular system (Williams 1987). These effects may be mediated by actions on A1 or A2 subtypes of the P1 purinoceptor (Burnstock 1983). Adenosine causes relaxation of isolated blood vessels from several species by an action on A2 receptors, but relatively few studies have been conducted using human vasculature. We have studied the effect of adenosine and some adenosine receptor agonists, namely 5-N-ethyl-carboxyamidoadenosine (NECA), R(-)-L-methyl-2-phenethyladenosine (L-PIA) and 2-chloroadenosine (C-ADO) on isolated umbilical arteries.

Lengths of umbilical cord were obtained shortly after normal deliveries. Umbilical arteries were dissected from the surrounding cord and mounted under 1g resting tension as intact rings in tissue baths containing 4ml physiological saline and aerated with 95% O_2 5% CO_2 at 37°C. Following 30 mins equilibration, arteries were exposed to physiological saline containing 125mM KCl. Vessels not contracting in response to this solution were not used for further studies. Following washout and recovery vessels were contracted by addition of 5-hydroxytryptamine (1uM) and adenosine receptor agonists added cumulatively once a stable tone was achieved. Relaxation was calculated as % reduction of induced tone. EC_{50} values are presented as geometric means (95% confidence limits) and maximum relaxation (max) as mean $\pm$ s.e. mean.

Agonist	EC_{50} (μM)	Max (%)	n
Adenosine	221 (48-1025)	88±7	10
NECA	156 (65-377)	100	6
L-PIA	393 (239-648)	98±3	10
C-ADO	348 (151-800)	100	8

In 34 segments adenosine or the adenosine receptor agonists, NECA, L-PIA, C-ADO caused concentration dependent relaxation. EC_{50} values derived from these data are shown in the table. However, in a further 46 segments adenosine receptor agonists failed to produce relaxation. Inosine (1μM - 1mM) failed to relax any umbilical artery segments (n = 8).

In conclusion adenosine and adenosine receptor agonists relax some segments of human umbilical artery. Although the agonist potency series NECA > Adenosine > L-PIA = C-ADO suggests that an A2 subtype of P1 purinoceptor may mediate these effects, the mechanism of action of these drugs in this tissue remains to be established.

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THE DIURETIC AND SALIURETIC PROPERTIES OF PD 116948, A POTENT ADENOSINE RECEPTOR ANTAGONIST

J.R. Keddie and M.G. Collis Bioscience II Dept, ICI Pharmaceuticals Div plc, Mereside, Alderley Park, Macclesfield, SK10 4TG.

In a previous study (Collis et al, 1986), we have shown that the adenosine antagonist 8-phenyltheophylline has diuretic, saliuretic and kaliuretic effects in the rat. PD 116948 (1,3 dipropyl-8-cyclopentyl xanthine) has been reported to be a potent adenosine receptor antagonist which differs from 8-phenyltheophylline in that it exhibits selectivity for the A1 sub-type in rat tissues (Haleen et al, 1987). However, other workers (Gurden et al, 1987) have questioned the A1 selectivity of this compound from studies using guinea-pig tissues. In the present study we have investigated the renal effects of PD 116948 in order to determine whether it exerts diuretic and saliuretic properties in the rat.

Paired Alderley Park Wistar rats, previously shown to be in the normal range for excretion of a saline load, were fasted overnight with free access to water. The rats were then given PD 116948 (i.v. or p.o.), its vehicle (3.3% DMSO, 0.25% 1M NaOH, in saline) or hydrochlorothiazide (p.o.) plus 40 ml/Kg of saline (0.9%, p.o.) before being placed in metaboles for urine collection over a 6 hour period. Urine volume and sodium, potassium and chloride concentrations were measured and the results were expressed as ml or mEq/100g body weight. PD 116948 at 0.1, 0.3 and 1.0 mg/Kg i.v. produced significant dose-related increases ($P<0.05$) in urine volume and in urinary sodium, potassium and chloride excretion (Table 1).

TABLE 1 The effect of PD 116948 on excretory parameters in the conscious rat.

Parmeter	Vehicle (n=5)	PD 116948 (mg/Kg). 0.1(n=5)	0.3(n=5)	1.0(n=4)
Urine Vol. (ml/100g)	3.51±0.15	4.47±0.17**	5.14±0.23***	5.7±0.19***
Urine Sodium (mEq/100g)	0.44±0.01	0.67±0.03***	0.71±0.03***	0.75±0.02***
Urine Potassium (mEq/100g)	0.095±0.009	0.119±0.005*	0.127±0.004*	0.153±0.018*
Urine Chloride (mEq/100g)	0.47±0.01	0.65±0.02***	0.69±0.03***	0.77±0.01***

(data expressed as mean ± s.e.m., *P<0.05, **P<0.01, ***P<0.001)

PD 116948 (1mg/Kg) also produced a significant increase in urine volume and in urinary sodium, potassium and chloride excretion ($P<0.002$) when administered by mouth to 5 pairs of rats, these effects were similar to that produced by 5mg/Kg (p.o.) of hydrochlorothiazide.

These results demonstrate that PD 116948 has diuretic, saliuretic and kaliuretic properties similar to those of a thiazide diuretic and to those previously reported for 8-phenyltheophylline. Further studies to examine the selectivity of the compound for A1 and A2 receptors in the rat are required in order to determine whether these effects on excretory function are due to selective antagonism at A1 receptors.

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THE ACTIONS OF THE ANTIANGINAL AGENT, BEPRIDIL, ON VOLTAGE-ACTIVATED SODIUM CURRENTS

Nicola. Y. Hather and Jeremy. J. Lambert. Neuroscience Research Group, Department of Pharmacology and Clinical Pharmacology, Ninewells Hospital and Medical School, Dundee, DD1 9SY.

Bepridil, an antianginal and antiarrhythmic agent, reduces the rate of rise (Vmax) of action potentials in guinea-pig atrial preparations, an action thought to be mediated via a block of voltage-activated sodium currents (Winslow and Kane, 1981). In addition, the effects of bepridil on Vmax have been reported to be use-dependent (Anno et al. 1984). We have used the modified patch clamp technique (Hamill et al. 1981) to investigate the actions of bepridil on voltage-activated sodium currents in bovine adrenomedullary chromaffin cells.

Sodium currents were activated by 10 ms voltage steps from -80 to 0 mV. Voltage-activated potassium currents were suppressed by dialysing cells with a caesium-based pipette solution and calcium currents were blocked by the inclusion of cobalt (1 mM) in the bathing medium. When 15 pulses were delivered at a frequency of 1 Hz, control sodium current amplitude decreased to 84 ± 2% (mean ± S.E.M., n=12) of that evoked by the first pulse. Bepridil (5 uM), applied externally, decreased the current elicited by the first pulse to 78 ± 2% of control. A further, use-dependent block, occurred during the train, such that the amplitude of the current activated by the fifteenth pulse was 59 ± 2% (n=6) of that evoked by the first pulse in the presence of bepridil. No significant use-dependent rundown was observed when the same concentration of bepridil was applied intracellularly. In contrast, external application of the lidocaine derivative QX-314 (0.5 mM) did not block the sodium current, while intracellular application of 0.5 mM QX-314 caused a rundown to 20 ± 8% (n=4) of the first current. Double pulse protocols were employed to explore the nature of the use-dependent block. Externally applied bepridil was found to shift the voltage-dependency of steady-state inactivation in the hyperpolarising direction, 5 uM bepridil causing a significant shift of -30 ± 4 mV, (n=4) in the midpoint of the h-infinity curve compared with control ($0.001>P$). Bepridil did not have a voltage-dependent action on the sodium current-voltage relationship.

Both the use-dependency of bepridil's action, and its effects on steady-state inactivation may be explained by a prolongation in the recovery time of the inactivated sodium current. When the interpulse interval was one second 92 ± 2%, (n=4) of the sodium current recovered from inactivation in control, however, the in the presence of 5 uM external bepridil the percentage recovery appeared smaller, 67 ± 5% (n=3). Intracellular dialysis with 1 mM N-bromoacetamide (NBA) partially prevented inactivation of the sodium current. In control, NBA-treated cells, there was no significant rundown of the non-inactivating current during a 1 Hz pulse train, whereas application of 5 uM external bepridil to these cells caused a decrease in the current amplitude to 47 ± 3% (n=4) of first pulse.

The results of this investigation support the reported actions of bepridil on cardiac sodium currents (Yatani et al. 1986), and examine further the mechanism of action. These actions may partly explain the efficacy of the drug in the treatment of cardiac arrhythmias.

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ELECTROPHYSIOLOGICAL EFFECTS OF PROPOFOL ON ISOLATED SINOATRIAL NODE PREPARATIONS AND ISOLATED ATRIAL CONDUCTION IN VITRO

I Briggs CG Heapy & Louise Pickering, Bioscience II Dept., ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, UK

Isolated cases of bradycardia have been seen during propofol anaesthesia (Langley & Heel, 1988); a study of the actions of the drug has thus been carried out in rabbit and guinea pig sinoatrial node preparations to assess effects on the cardiac pacemaker.

The rabbit or guinea pig sinoatrial node area was isolated and pinned to the floor of a small dish, superfused with Tyrode's solution gassed with 95% O_2 / 5% CO_2, at 37°C. Spontaneous intracellular action potentials were recorded using conventional techniques. The potentials were amplified, and digitised for measurement and analysis by microcomputer. Propofol in its clinical form (Diprivan) as an emulsion in soya oil, or its vehicle was applied by perfusion through the dish at 2 ml. per minute in a range of dilutions in Tyrode's solution. Repeated measurements were made of the action potential parameters for comparison with control measurements.

The effects of propofol were also determined on conduction time in isolated guinea pig atria. Propofol was applied by perfusion to three pairs of atria in successive concentrations of 10, 33, and 100 µg/ml. Two pairs of atria were treated simultaneously with similar dilutions of vehicle as controls. Conduction times (CT) were determined by digital oscilloscope measurements of the interval between stimulus artefacts evoked by stimuli applied to one end of the preparation, and extracellular evoked potentials recorded from the other end.

The effects of propofol on sinoatrial nodes were relatively slight at the concentrations likely to be seen in plasma in vivo (anaesthetic levels are 3 - 8 µg/ml. The effects on the spontaneous beating rate were slight at 1µg/ml or lower concentrations; at higher concentrations, small effects on rate were observed which in the rabbit node were principally related to slowing of the upstroke of the action potential, and in the guinea pig to slowing of diastolic depolarisation. In the rabbit, these effects resembled those due to membrane stabilisation by local anaesthetics and verapamil. In the guinea pig the effects were different in that phase 0 depolarisation was enhanced rather than depressed. Thiopentone had little effect in the guinea pig node at 30 µg/ml, but depressed activity at 100 µg/ml. The vehicle used for propofol had no effects at 1/100 dilution.

Propofol had only small effects on atrial conduction at 10 µg/ml (7.8% increase in CT) but severely slowed conduction at 33 µg/ml (41% increase in CT) and caused complete conduction block at 100 µg/ml in all three tissues. The vehicle had no effects on CT at equivalent dilutions.

Propofol thus had bradycardic activity and membrane stabilising actions at high concentrations, which are unlikely to be used clinically. Since the bradycardia observed clinically is reversible by administration of atropine, it is probably due to a centrally mediated increase in vagal tone.

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POTASSIUM DEPOLARIZATION SELECTIVELY BLOCKS POSITIVE INOTROPIC EFFECTS OF SODIUM CHANNEL ACTIVATORS IN GUINEA-PIG ATRIA

H. Berthold and G. Scholtysik, Preclinical Research, Sandoz Ltd., 4002 Basle, Switzerland

Drugs which increase myocardial contractility comprize a heterogenous group of compounds with widely differing mechanisms of action, including inhibition of the Na^+/K^+-ATPase, increase of cAMP levels either by stimulation of β-adrenoceptors or inhibition of cAMP-phosphodiesterase (PDE) and activation of cardiac calcium channels. Cardioselective activators of sodium channels have recently been shown to represent a new class of positive inotropic agents, of which the prototype is DPI 201-106 (Scholtysik *et al.*, 1985; Scholtysik, 1989). The aim of the present study was to evaluate a simple test procedure for recognition of sodium channel activating mechanisms of cardiotonic compounds.

We investigated various drugs with the above mentioned mechanisms of action by comparing their effects in normal and in potassium-depolarized isolated guinea-pig left atria. The atria were mounted in a modified Krebs-Henseleit solution (2.5 mM Ca^{2+}) and were electrically stimulated at 1 Hz. $[K^+]_0$ was 4.7 mM and 22 mM, respectively, in the two series of experiments. Concentration-response curves were established by cumulatively increasing the drug concentration every 15 min. Potassium depolarization had minimal effects on basic force of contraction *per se*.

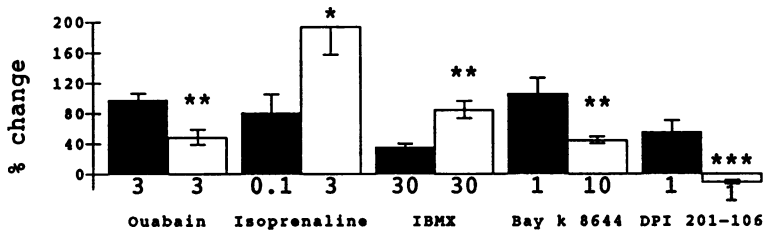

Figure 1. The maximal force of contraction (mean ± SEM; N=6 per group) in per cent change from baseline developed by compounds representative of different classes of positive inotropic agents. The filled bars represent atria in normal $[K^+]_o$ (4.7 mM) and the open bars are depolarized preparations ($[K^+]_o$ 22 mM). The figures denote the drug concentrations (μM) at which the maximal response was observed. (*p<0.05, **p<0.01, ***p<0.001; unpaired two-tailed t-tests).

Depolarization decreased the positive inotropic effect of ouabain to about half-maximal values. The effects of isoprenaline and of the PDE inhibitors, IBMX, amrinone and OPC 8212 were increased more than twofold or remained unchanged (OPC 8212). The effects of the calcium channel activators, Bay k 8644, CGP 28 392 and SDZ 202-791 were decreased twofold and the effects of the sodium channel activators, DPI 201-106, SDZ 210-921, veratridine and ATX II were completely abolished.

Partial depolarization of the membrane of the myocardial cell from about -90 mV at resting state to about -50 mV by increasing $[K^+]_0$ to 22 mM inactivates the fast sodium channels (Pappano, 1970). Therefore, drugs which exert their positive inotropic action through activation of these channels become ineffective under these conditions. The results suggest that partial depolarization is a suitable model to identify sodium channel activators and to distinguish them from compounds with other mechanisms of action.

Drugs used in the present study: DPI 201-106 ((S)-(-)-4-[3-(4-diphenylmethyl-1-piperazinyl)-2-hydroxypropoxy]-1H-indole-2-carbonitrile), IBMX (3-isobutyl-1-methylxanthine), OPC 8212 (3,4-dihydro-6-[4-(3,4-dimethoxybenzoyl)-1-piperazinyl]-2(1H)-quinolinone), Bay k 8644 (methyl 1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoromethylphenyl)-pyridine-5-carboxylate), CGP 28 392 (4-(2-difluoromethoxyphenyl)-2-methyl-5-oxo-1,4,5,7-tetrahydro-furo[3,4-b]pyridine-3-carboxylic acid ethyl ester), SDZ 202-791 (isopropyl (4-(2,1,3-benzoxadiazol-4-yl)-1,4-dihydro-2,6-dimethyl-5-nitro-3-pyridine carboxylate), SDZ 210-921 ((R)-(+)-4-[3-(4-diphenylmethyl-1-piperazinyl)-2-hydroxypropylamino]-1H-indole-2-carbonitrile) and ATX II (*Anemonia Sulcata* toxin II).

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EFFECT OF INSULIN ON ADRENERGIC RESPONSES OF ISOLATED CARDIAC TISSUES FROM CONTROL AND DIABETIC RATS

C.E. Austin, A.M. Breckenridge & R. Chess-Williams, Department of Pharmacology & Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.

Diabetes of short duration in the rat is associated with an increase in cardiac β-adrenoceptor sensitivity (Austin et al., 1987). Lee and Downing (1976) have shown that inclusion of insulin in the bathing medium may alter the responsiveness of isolated cardiac tissues to noradrenaline. We have therefore investigated whether the presence of insulin can reverse the β-adrenoceptor supersensitivity seen with isolated tissues from diabetic animals.

Female Wistar rats were made diabetic by a single intraperitoneal injection of streptozotocin (50mg kg^{-1}). Controls were age-matched. After 14 days, left atria (LA) and papillary muscles (PM) were removed and set up in aerated krebs solution at 37°C. Tissues were paced at 1Hz and isometric tension recorded. Tissues were incubated for 1 hour with or without Humulin (5 uml^{-1}). Cumulative concentration-response curves to isoprenaline were then constructed in the presence of uptake blockers. The presence of insulin on the responses to phenylephrine, forskolin and calcium was also investigated in tissues from diabetic animals.

Table 1 : EC_{50} values with 95% confidence limits for isoprenaline and forskolin (n≥5)

Tissue	Insulin absent		Insulin present	
Controls	Isoprenaline(nM)	Forskolin(uM)	Isoprenaline(nM)	Forskolin(μM)
LA	121.6(39.6-373.7)	-	211. (56.4-789.7)	-
PM	147.5(63.4-343.2)	-	523.2(138.2-1980.6)	-
Diabetics				
LA	3.8 (1.3-114)*	165.5(46.9-583.7)	45.5(14.4-143.6)*	152.5(23.5-987.2)
PM	15.2 (7.4-31.0)	337.2(118.9-956.6)	197.4(56.3-692.3)*	839.5(244.7-2447.2)

* $P < 0.005$ compared with insulin absent.

In diabetic animals cardiac sensitivity to isoprenaline was increased and EC_{50} values were significantly reduced (Table 1) compared with age-matched controls ($P < 0.001$ for both LA and PM). The presence of insulin in the bathing medium increased isoprenaline EC_{50} values in control and diabetic tissues (Table 1). The change in sensitivity however, was greater and only statistically significant in tissues from diabetic animals. In the presence of insulin, there was no significant difference between the isoprenaline EC_{50} values for tissues from control and streptozotocin-treated animals.

The presence of insulin did not affect the sensitivity of cardiac tissues from diabetic animals to forskolin (Table 1). The responses of these tissues to phenylephrine and calcium were similarly unaffected by insulin. The EC_{50} values for phenylephrine in tissues from diabetic animals in the absence [LA, 0.8 (0.4-1.6)μM; PM, 1.3 (0.5-3.0)μM] and presence [LA, 1.2 (0.3-4.3)μM; PM, 2.5 (1.2-5.4)μM] of insulin were similar as were the EC_{50} values for calcium chloride in the absence [LA, 7.6 (4.5-12.7) mM; PM, 6.6 (4.3-10.3)mM] and presence [LA, 10.0 (6.6-15.1)mM; PM, 11.0 (8.1-14.9)mM] of insulin.

These results suggest that insulin may depress cardiac responses to β-adrenoceptor agonists but not forskolin. Also, the presence of insulin in the bathing medium can reverse the cardiac β-adrenoceptor supersensitivity induced by diabetes.

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EFFECTS OF ANTIANGINAL AGENTS ON EPICARDIAL ST-SEGMENT ELEVATION DURING TEMPORARY CORONARY ARTERY OCCLUSION IN ANAESTHETISED DOGS

R. B. Jones and G. A. Foster introduced by M. F. Sim, Research Department, The Boots Company PLC, Nottingham, NG2 3AA

Elevation of the epicardial ECG ST-segment following temporary coronary artery occlusion has been used as an index of myocardial ischaemia (Maroko et al., 1971). This study was conducted to determine whether a variety of antianginal agents were effective in reducing ST-segment elevation in a temporary coronary artery occlusion model of myocardial ischaemia.

Pentobarbitone anaesthetised dogs were subjected to 2 minute periods of coronary occlusion every 30 minutes. The first 2 occlusions acted as controls and the following 3 occlusions recorded the effect of treatment. Elevation of the epicardial ST-segment was measured before and 7 times during the occlusion period and the area under the ST-segment curve (ST-Area) calculated. The dose response of propranolol (P, 0.1-1.0 mg/kg i.v.), nifedipine (N, 0.01-0.10 mg/kg i.v.) verapamil (V, 0.03-0.3 mg/kg i.v.), bepridil (B, 1-6 mg/kg i.v.) and glycerol trinitrate (G, 20-80 µg/kg i.v.) n = 4 per group were constructed and compared to a saline dosed control group. Drugs were bolus dosed 5 minutes prior to coronary artery occlusion except for G which was administered as a slow infusion over 5 minutes.

P caused a significant reduction ($P < 0.05$) in the ST-Area of 58, 71 and 76% at 0.1, 0.3 and 1.0 mg/kg i.v. respectively compared to pre-dose control values. B (1.0, 3.0 and 6.0 mg/kg i.v.) and V (0.03, 0.10 and 0.3 mg/kg i.v.) caused a reduction of 17, 46 and 64% and 15, 49 and 54% in the ST-Area, only the highest two doses in each case being significantly different ($P < 0.05$) from pre-dose values. G caused a slight reduction in ST-Area of 21, 32 and 21% at 20, 40 and 80 µg/kg i.v. respectively but this reduction was not significantly different from pre-dose control values. N caused a worsening of ischaemia increasing ST-Area by 20, 12 and 23% at 0.01, 0.03 and 0.1 mg/kg i.v. respectively.

The data do not agree entirely with the literature reports utilising ST-segment analysis during coronary occlusion. Constantin et al (1983) observed V, B and N to be effective and Theroux et al, (1974) demonstrated that G is anti-ischaemic. Campbell and Meier (1983) also showed that N and a beta blocker metoprolol were effective. The reason why G and N were ineffective in this study is uncertain but may have been due to the tachycardia they produce in anaesthetised dogs. Certainly only those compounds which showed a significant bradycardic component to their cardiovascular activity were effective in this model of myocardial ischaemia.

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REGIONAL ISCHAEMIA DOES NOT INDUCE LEUKOTRIENE RELEASE FROM RAT ISOLATED PERFUSED HEART

G. Wright, M.A. Trevethick and P. Strong, Department of Peripheral Pharmacology, Glaxo Group Research, Ware, Herts, SG12 ODP, UK.

The calcium ionophore, A23187, but not global ischaemia, induces leukotriene (LT) release from the rat isolated perfused heart (Trevethick et al, 1987). In this study, we have investigated whether regional ischaemia and reperfusion in vitro stimulates LT release.

Hearts were perfused at constant flow by the Langendorff method (Kennedy et al, 1983). After 30 mins equilibration, the left coronary artery (LCA) was ligated for 30 or 120 mins and then reperfused. Coronary effluent samples were collected throughout and assayed for lactate dehydrogenase (LdH) activity (Bergmeyer et al, 1974), lactate efflux (Gawehn et al, 1974), and for the prostacyclin metabolite, 6-keto-$PGF_{1\alpha}$ (6-$KPGF_{1\alpha}$) and peptidyl LTs ($C_4/D_4/E_4$) by specific radioimmunoassay. The results are summarised in Table 1.

Table 1. Eicosanoid and enzyme efflux induced by LCA occlusion/reperfusion (mean ± SEM, n=3)

	Occlusion (mins)	Pre-Occlusion	Occlusion	Reperfusion (mins) 0-5	5-10
$LTC_4/D_4/E_4$	30	0.4±0.2	0.5±0.1	0.2±0.2	0.6±0.1
(ng/min)	120	0.2±0.1	0.4±0.1	0.6±0.3	0.5±0.4
6-$KPGF_{1\alpha}$	30	0.6±0.1	2.2±0.1	4.4±0.4	1.7±0.3
(ng/min)	120	0.3±0.1	1.0±0.4	1.1±0.2	1.3±0.3
LdH	30	0±0	0.1±0.0	0.2±0.1	0.4±0.1
(IU/min)	120	0±0	0.1±0.0	2.2±1.3	1.5±0.6
Lactate	30	1.1±0.3	0.9±0.1	2.3±0.4	0.3±0.1
(μmol/min)	120	1.0±0.3	0.7±0.1	0.4±0.2	0.7±0.2

Occlusion for both 30 and 120 min increased 6-$KPGF_{1\alpha}$ release, but had no significant effect on the other parameters. Reperfusion, following 30 mins of occlusion, further increased the release of 6-$KPGF_{1\alpha}$ and stimulated lactate efflux, but not that of LT or LdH. LdH release was observed following prolonged periods (120 min) of occlusion and reperfusion, but again there was no effect on LTs.

The inability of rat hearts to release LTs in response to regional ischaemia and reperfusion parallels our previous experience using global ischaemia (Trevethick et al, 1987). Since the effect of A23187 clearly demonstrates that rat cardiac tissue has the potential to synthesise LTs, it seems likely that ischaemia and reperfusion in vitro is simply an ineffective stimulus for LT release. As yet, factors regulating the synthesis and release of LTs by cardiac muscle remain poorly understood.

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REPERFUSION-INDUCED INJURY: STUDIES OF INFARCT SIZE LIMITATION BY ANTI-FREE RADICAL INTERVENTIONS IN THE RAT HEART

R. Crome & A.S. Manning, Department of Biology, Roche Products Ltd, P.O. Box 8, Welwyn Garden City, Hertfordshire, AL7 3AY

Recent studies have suggested that anti-free radical agents may limit ultimate myocardial infarct size. The two most important sources of free radicals are thought to be xanthine oxidase (XO) and activated neutrophils. Treatment with an XO inhibitor (1), or anti-oxidant enzymes (2) or inhibitors of neutrophil accumulation (3) have been shown to limit infarct size in a canine preparation.

In this study we have compared two XO inhibitors, allopurinol and amflutizole, benoxaprofen which inhibits neutrophil accumulation, DMTU (a scavenger of hydroxyl ions and peroxides) and superoxide dismutase (SOD), conjugated to PEG or in combination with catalase, in their ability to limit infarct size in the rat. Anaesthetised rats underwent 20 min occlusion (O) of the left anterior descending coronary artery followed by 60 min reperfusion (R). The hearts were then removed, frozen, and infarct size assessed using a single blind procedure where hearts were sliced, stained with nitro blue tetrazolium and the necrotic area dissected, weighed and expressed as percentage of ventricular tissue.

Table 1. Effects of anti-free radical interventions on infarct size

	Mortality(%)	Infarct size (% vent. tissue)
Vehicle (NaOH + saline) i.v. 15 min prior O	7/26 (27)	22.2 ± 2.1
Allopurinol (20 mg/kg i.v. 15 min prior O + 20 mg/kg p.o. 24 hr prior O)	7/22 (32)	17.9 ± 2.9
Allopurinol (100 mg/kg i.v. 15 min prior O)	4/20 (20)	20.3 ± 3.4
Amflutizole (50 mg/kg i.v. 15 min prior O)	3/17 (18)	22.7 ± 2.6
Amflutizole (100 mg/kg i.v. 15 min prior O)	3/15 (20)	24.1 ± 2.5
Vehicle (PEG:CMC) for 3 days prior O	5/17 (29)	27.6 ± 2.6
Benoxaprofen (30 mg/kg p.o. for 3 days prior O)	1/12 (8)	13.9 ± 2.6**
Vehicle (saline) { infused for 45 min	6/22 (27)	20.9 ± 2.6
DMTU (500 mg/kg i.v. { starting 15 min prior O	1/11 (8)	13.4 ± 1.8*
Vehicle (saline) i.v. 5 min prior O	8/22 (39)	26.7 ± 2.6
SOD in PEG (15,000 units/kg i.v. 5 min prior O)	7/24 (29)	21.5 ± 2.7
SOD (35,000 units/kg) + catalase (400,000 units/kg) { i.v. 5 min prior R	0/9 (0)	26.4 ± 3.0

*P<0.05; **P<0.002 versus appropriate control;

No beneficial effects were observed with xanthine oxidase inhibitors, or administration of SOD with or without catalase. Infarct size limitation was observed following pretreatment with benoxoprofen which prevents the accumulation of activated neutrophils (as shown by us previously (4)) and also by the administration of a hydroxyl radical scavenger. These findings indicate that infarct size limitation, as assessed by tetrazolium staining, can be achieved by certain anti-free radical interventions in this preparation whilst XO inhibition does not appear to be effective.

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PHARMACOLOGICAL RESPONSIVENESS OF HUMAN CORONARY ARTERIES: RELATIONSHIP TO AGE AND DISEASE

L. Hyland, I. Smeeton' & D.M. Paton, Department of Pharmacology and Clinical Pharmacology and 'Department of Pathology, University of Auckland, Auckland, New Zealand.

Atherosclerotic human coronary arteries have been shown to be more sensitive to certain agonists than normal arteries (Ginsburg et al., 1981, Endo et al., 1976). However, studies involving human coronary arteries are complicated by the fact that atherosclerosis is at least partially age-related. We have examined the effect of age and disease on receptor-mediated and non-receptor-mediated responsiveness of the right human coronary artery obtained post mortem. Ring segments were placed in organ baths between a fixed rod and a force transducer under 1.5g tension and bathed in Krebs-Henseleit solution at 37°C. Arteries responded to noradrenaline by contraction, relaxation or a biphasic concentration-response curve consisting of both contraction and relaxation. The % of coronary arteries responding to noradrenaline by relaxation decreased in an age-related manner until the dominant response became contraction. The differential responsiveness to noradrenaline correlated more closely with the age of the individual rather than the degree of disease of the vessel. We examined beta-1 and beta-2-adrenoceptors separately, as the relaxation elicited by increasing concentrations of isoprenaline following contraction by 20 μm KCl in the presence of prazosin (0.1 μm) and ICI 118 551 (0.01 μm) to examine beta-1-adrenoceptors and atenolol (0.3 μm) to examine beta-2-adrenoceptors. Both beta-1 and beta-2-adrenoceptors from those aged < 45 years showed a significantly greater ability to mediate relaxation to isoprenaline than those aged > 45 years. Mean maximum beta-1-mediated relaxation was 54% ± 10.3% and 11% ± 14.5% respectively ($P < 0.05$, $n = 5$ each). Mean maximum beta-2-mediated relaxation was 50% ± 10% and 10.5% ± 5.8% respectively ($P < 0.01$, $n = 5$ or 6). There was a small but significant negative correlation between the potency of phenylephrine in terms of EC50 values and age ($r = -0.524$, $n = 18$, $P < 0.05$). No significant disease-related changes in beta-1, beta-2 or alpha-1-mediated responsiveness were observed. Neither aging nor degree of disease affected the non-receptor-mediated contractions to KCl, implying that non-specific functional changes were not responsible for the alteration in adrenergic responsiveness. Sodium nitroprusside exhibited an age and/or disease-related loss of potency over the concentration range 0.1 - 0.3 μm following contraction by 40 μm KCl. Mean relaxation in those > 50 years was 5% ± 4.5% and 18.4% ± 8.5% at 0.1 and 0.3 μm respectively (all samples moderately/severely diseased) compared with 38.5% ± 13.7% and 60.3% ± 14.6% in those < 40 years (all samples were normal).

Our results show a loss of beta-adrenoceptor-mediated vasodilatation and a decline in alpha-1-adrenoceptor sensitivity with increasing age in the human coronary artery. These alterations were apparently unaffected by the degree of disease present. This study emphasises the importance of the age factor in studies comparing normal and atherosclerotic human coronary arteries.

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UPTAKE OF [^{14}C]-INDAPAMIDE BY RAT TISSUES: AN IN VITRO STUDY

P.J. Nicholls, Welsh School of Pharmacy, UWCC, PO Box 13, Cardiff CF1 3XF.

There is evidence (Chaffman et al. 1984) that the antihypertensive action of indapamide is the result of not only a weak diuresis, but also of a decrease in vascular reactivity and peripheral vascular resistance. In view of an earlier observation (Campbell et al. 1977) that indapamide accumulates in vascular smooth muscle of the rat, the present investigation has examined the in vitro uptake of radioactivity by pieces of rat aorta (A), diaphragm (D) and heart (H) incubated in a medium containing ^{14}C-indapamide.

Male albino rats (120-200g) were killed by stunning and exsanguination and the A, D and H were rapidly excised and washed in Krebs-Ringer bicarbonate solution (KRB) pH 7.2, 4^{o}C. A and D were cut into sheets of about 0.5mm^{2}, while muscle from the apex of the H was cut into rods (0.5 x 0.5 x 1.0mm). These tissues (20mg) were incubated at 36.5^{o}C in KRB (5ml) containing ^{14}C-indapamide. When inhibitors were employed a pre-incubation of 15 min with tissue occurred. Uptake of radioactivity was assessed as the tissue-to-medium ^{14}C concentration ratio (T/M).

For the 3 tissues (incubated in 0.46μM indapamide), T/M rose with time to a plateau at 30 min. However, these max values (n=5) were significantly ($P<0.05$) greater for A (7.9±0.6) than for either D (3.0±0.2) or H (3.8±0.4). The values were not significantly different when T/M was determined with tissues from spontaneously hypertensive rats (7.1±1.4, 2.9±0.2, 4.6±0.6 for A, D and H respectively; n=5). T/M (30 min) was independent of indapamide concentration (0.05-20.0 M), values ranging between 7.0±1.8 and 8.0±1.1 (n=5) for A. Uptake was temperature dependent; comparing incubations at 36.5 and 10^{o}C, the T/M was reduced from 7.3±1.3 to 4.0±1.3 for A and from 4.1±0.1 to 2.5±0.2 for H. Despite this, KCN (1mM) did not influence uptake of ^{14}C by either A or D suggesting that the process was not dependent on energy production. Imipramine (Chi and Dixit 1977) and Na^{+}-depletion (Dollery and Junod 1976) can inhibit uptake of cationic drugs into lung, possibly by interfering with an Na^{+}-dependent process. However, neither imipramine (0.1mM) nor replacement of Na^{+} by Li^{+} in KRB had any influence on uptake of ^{14}C-indapamide into A or D. Uptake of indapamide by erythrocytes is associated with binding to carbonic anhydrase (Campbell et al. 1977). The carbonic anhydrase inhibitor, acetazolamide (50mM) was unable to alter the T/M of indapamide for either A or D. Arteries are elastin-rich (Rucker and Tinker 1977) and the binding of indapamide (0.5μM) to elastin compared to collagen was examined by the T/M. After 30 min incubation, the values were 40.5±4.4 and 5.7±0.7 for elastin and collagen (20mg/5ml) respectively.

The results suggest that uptake of indapamide by A, D and H in the rat is a passive process. Binding of the drug to A does not involve carbonic anhydrase but may be associated with elastin.

^{14}C-Indapamide was kindly supplied by Servier R and D, Slough.

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EFFECTS OF XAMOTEROL ON THE HEART IN ANAESTHETISED DOGS TREATED WITH EITHER PENTOLINIUM OR SYROSINGOPINE

S.J.G. McAuliffe, A.A. Oldham and H.M. Snow. Bioscience Department II, ICI Pharmaceuticals, Mereside, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Xamoterol is a beta$_1$ adrenoceptor partial agonist. In dogs depleted of catecholamines by pretreatment with syrosingopine (syro) the intrinsic sympatho-mimetic activity (ISA) is 43% relative to the effects of isoprenaline. (Nuttall & Snow, 1982). In the present study, the effects of xamoterol on heart rate (HR) in dogs pretreated with syro were compared with effects on HR and the maximum rate of rise of left ventricular pressure (dp/dt max) in dogs pretreated with the ganglion blocker pentolinium (pent).

Alderley Park beagle dogs of either sex were allocated to pent or syro groups. The latter group received syro chronically, 2.5mg/kg s.c. daily for two days followed by a third dose on the morning of the experiment. Both groups of dogs were anaesthetised using sodium pentobarbitone and artificially respired. In pent dogs a balloon catheter was introduced into the thoracic aorta and a catheter tipped pressure transducer introduced into the left ventricle. In one group of pent dogs a pacing electrode was introduced into the right atrium. In all dogs the blood pressure was monitored. HRs were measured from ECG. Pent dogs received acutely pent 2mg/kg iv plus atropine 2mg/kg iv prior to each dose response curve (DRC). A control isoprenaline (iso) DRC was followed by a full DRC to xamoterol prior to a second iso DRC. In syro dogs the initial iso DRC was followed by a series of iso DRC's in the presence of increasing doses of xamoterol.

HR ED_{50}s in syro and pent dogs and dp/dt max ED_{50} in unpaced dogs were similar. Pacing caused a small but significant reduction in dp/dt max ED_{50}. A similar pattern was observed with dp/dt max K_Bs, although the effect of pacing did not reach statistical significance. ISAs obtained were consistent throughout (table).

Treatment		Heart Rate			dp/dt max		
		ED_{50} (μg/kg)	K_B (μg/kg)	% ISA	ED_{50} (μg/kg)	K_B (μg/kg)	% ISA
Syro	Iso	0.4±0.09					
N=4	Xamoterol	2.5±0.05	8.7±0.5	47.5±4.3			
Pent	Iso	0.31±0.1			0.24±0.1		
N=4	Xamoterol	2.4±0.7	7.1±1.7	43.1±3.8	4.0±0.6	7.2±1.9	43.1±6.9
nonpaced							
Pent	Iso				0.11±0.01		
N=4	Xamoterol				1.8±0.5*	3.6±0.7	46.0±1.8
paced							

*$p<0.05$ compared with paced group

In conclusion the partial agonist profile of xamoterol was similar in the syro and pent groups. In the pent model partial agonist effects of xamoterol on dp/dt max were similar to those on HR.

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PHORBOL-12,13-DIBUTYRATE INHIBITS ATP- BUT NOT A23187-INDUCED EDRF RELEASE FROM CULTURED PIG AORTIC ENDOTHELIAL CELLS

J.A. Smith, Department of Cardiology, University of Wales College of Medicine, Heath Park, Cardiff, CF4 4XN

Endothelium-derived relaxing factor (EDRF) is known to elevate cyclic GMP levels in the endothelial cells from which it is released, and this can be used as a measure of EDRF release (Smith, 1989). Phorbol-12,13-dibutyrate (PDB) has been shown to inhibit acetylcholine and substance P-induced EDRF release from rings of rabbit aorta (Lewis and Henderson, 1987) or cultured pig aortic endothelial cells (Smith, 1989). This study examines the effects of PDB on EDRF release from cultured pig aortic endothelial cells induced by ATP or the calcium ionophore A23187. Experimental methods were as described previously by Smith (1989). Cyclic GMP was measured by radioimmunoassay and DNA assayed by the method of Kissane and Robins (1958).

A23187 (10^{-7}M, for 3 min) induced a 6.3 fold increase in the cyclic GMP content of primary cultures of pig aortic endothelial cells, from a resting level of 18.7±1.5 fmol μg^{-1} DNA (n=12) to 117.5±11.3 fmol μg^{-1} (n=6, P<0.001). Pretreatment for 20 min with the EDRF inhibitor haemoglobin (Hb, 10^{-5}M) prevented the A23187-induced increase in cyclic GMP and reduced the cyclic GMP content of the cells to below resting levels (11.6±0.8 fmol μg^{-1} DNA, n=6, P<0.005 cf resting levels). Pretreatment with PDB (3 x10^{-7}M, 5 min) inhibited (P<0.05) the A23187 (10^{-7}M)-induced increases in cyclic GMP (78.6±9.3 fmol μg^{-1} DNA, n=6) when compared with pretreatment with the inactive 4α-phorbol-12,13-didecanoate (PDD, 3x10^{-7}M, 5 min, 117.8±13.8 fmol μg^{-1} DNA, n=6), which did not differ from controls. The inhibitory effect of PDB on A23187-induced increases in endothelial cell cyclic GMP was not observed in a separate experiment in which superoxide dismutase (SOD, 30 u ml^{-1}) and catalase (CAT, 100 u ml^{-1}) were added simultaneously with 3x10^{-7}M PDB 5 min prior to the addition of 10^{-7}M A23187 (3 min). The cyclic GMP levels were found to be (fmol μg^{-1} DNA): resting, 12.6±1, n=9; A23187, 20.9±1.9, n=9 (P<0.005 cf resting); A23187+PDB, 22.3±2.7, n=9 (P<0.6 cf A23187). ATP (10^{-5}M for 3 min) in the presence of SOD (30 u ml^{-1}) and CAT (100 u ml^{-1}) induced a 2.8 fold increase in endothelial cells cyclic GMP content from 19.4±2.3 (n=6) to 55.1±3.4 fmol μg^{-1} DNA (n=6). This increase was prevented by 20 min Hb (10^{-5}M) pretreatment (4.5±0.8, fmol μg^{-1} DNA, n=5, p<0.005 cf resting), was not affected by PDD (3x10^{-7}M, 5 min) pretreatment (3 fold, 58.5±10.1 fmol μg^{-1} DNA, n=5), but was inhibited by PDB (3x10^{-7}M, 5 min) pretreatment (1.5 fold, 28.3±5.8 fmol μg^{-1} DNA, n=6, P<0.05 cf PDD pretreatment).

It is concluded from these results that activation of protein kinase C can inhibit receptor mediated release and/or production of EDRF by ATP, but does not inhibit the receptor-independent release of EDRF induced by A23187. The apparent inhibition of A23187-induced release by PDB in the absence of SOD and CAT is due to enhanced EDRF breakdown by superoxide anions, production of which is synergistically enhanced by phorbol esters and calcium ionophores (Matsubara and Ziff, 1986).

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TRANSPORT OF L-ARGININE, A POSSIBLE PHYSIOLOGICAL PRECURSOR OF EDRF, BY BOVINE ENDOTHELIAL CELLS

J.M. Sneddon, T.M. Bearpark, J.R. Vane. The William Harvey Research Institute, St. Bartholomew's Hospital Medical College, Charterhouse Square, London EC1M 6BQ.

The vascular endothelium releases endothelium derived relaxing factor, EDRF, which accounts for the endothelium dependent relaxation produced by a wide variety of vasodilators. The biosynthetic pathways for EDRF are unknown, but recent evidence suggests that EDRF is nitric oxide (NO) which may be derived from the terminal guanidino nitrogen atoms of L-arginine arising from metabolism of either arginine-containing peptides (Thomas and Ramwell, 1988) or free arginine (Palmer et al., 1988; Schmidt et al., 1988). Little is known about the transport and metabolism of L-arginine by endothelial cells. The aim of the present study was to investigate the transport of L-arginine, the putative precursor of EDRF, by endothelial cells.

Bovine aortic endothelial cells (BAEC) were subcultured in 6-well tissues culture plates. At confluence (10^6 cells/well) the monolayers were washed with Hanks Balanced Salt Solution (HBSS) and then incubated at 36°C with the appropriate concentrations of L-arginine or its analogues dissolved in HBSS containing a tracer concentration of H^3-L-arginine. After an appropriate time interval (usually 20 min) the monolayers were washed with HBSS and extracted with 1ml ice cold methanol. Aliquots were taken for scintillation counting, and L-arginine accumulation determined as μmoles/10^6 cells/20 min.

In our experiments L-arginine transport by BAEC exhibited the following characteristics:

1. It occurs by a non-saturable mechanism resolvable into two components
   a) a high affinity low capacity system at concentrations below 100μM (apparent Km 0.52μM; Vmax 1.58μm/10^6 cells/20min)
   b) a linear non-saturable uptake, the kinetic characteristics of which have not been determined.
2. Uptake is not Na^+-dependent, and is enhanced by incubating BAEC in arginine free medium.
3. The uptake mechanism is relatively selective for L-arginine and is not inhibited by the following arginine analogues (final conc. 100μM); D-arginine, DL-C-propargylglycine, L-ornithine, L-arginine methylester, Na-L-benzoyl-L-arginine methyl ester, N^G-monomethyl-L-arginine, L-citrulline. Partial inhibition of uptake occurs with L-homoarginine and L-canavanine.
4. After 20 min incubation over 95% of the radiolabel was extractable as H^3-L-arginine, with a small variable amount of H^3 co-chromatographing with L-citrulline.

The transport of L-arginine by BAEC has similarities to the transport system for cationic amino acids found in other cells and designated system Y^+ (White, 1985). L-arginine enters BAEC rather slowly and the majority of the accumulated arginine is probably metabolised by pathways other than that leading directly to EDRF.

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THE NOVEL 5-HT_2 ANTAGONIST ICI 169,369 CAN CAUSE AN ENDOTHELIUM-DEPENDENT RELAXATION OF RABBIT AORTA

J.G.P. Pires, M. Jacobs & A.G. Ramage, Academic Department of Pharmacology, Royal Free Hospital School of Medicine, Rowland Hill St, Hampstead, London NW3 2PF.

The novel 5-HT_2 antagonist ICI 169,369 (Blackburn et al., 1988), at high doses, differed in its action in the anaesthetised cat, when compared with other 5-HT_2 antagonists, in that it caused a transient fall followed by a rise in blood pressure (Ramage, 1988). The present experiments in rabbit aorta were carried out to investigate whether this in vivo response could be accounted for by a direct action on blood vessels.

Thoracic aortae from male New Zealand White rabbits (2-2.5 kg) were cut transversely into 2mm wide rings. Paired intact rings and endothelium-denuded rings, prepared as previously described (Andrews et al. 1987), were suspended under a resting tension of 2g in a 16 ml jacketed organ bath containing modified Krebs solution (NaCl 118 mM; KCl 4.8 mM: $CaCl_2$ 2.5 mM, $MgSO_4$ 1.2 mM, KH_2PO_4 1.2 mM, $NaHCO_3$ 24 mM, NaEDTA 0.03mM and glucose 11 mM), bubbled with 95% O_2 5% CO_2, at 37 °C. After an equilibration period of 90 min, the tissues were precontracted by phenylephrine (0.3 μM) or $PGF_{2\alpha}$ (1-3 μM) and the presence of endothelium was assessed by relaxation to cumulative additions of acetylcholine. The tissues were then washed with Krebs alone for 40-50 min, recontracted and a cumulative dose response curve to ICI 169,369 (1-50 μM) was obtained. This was repeated after washing and pre-incubation with either indomethacin (1h) or an antagonist (30 min).

ICI 169,369 caused a dose-dependent relaxation in intact rings precontracted with $PGF_{2\alpha}$ or with phenylephrine. The threshold concentration for the response was 1 μM and maximal relaxation was achieved at 30 μM, above which concentration the tissues recontracted. The maximum relaxation was reversed by methylene blue (10 μM) or haemoglobin (0.1 μM) but not inhibited by indomethacin (10 μM). Pre-incubation with methiothepin (1 μM), MDL 72222 (1 μM), ketanserin (0.05 μM), Wy 26392 (1 μM), prazosin (1 μM), propranolol (5 μM), mepyramine (0.1 μM), cimetidine (1 μM), atropine (0.1 μM) or theophylline (5 μM) were without any effect on the relaxation. ICI 169,369 failed to evoke a contraction in endothelium-denuded rings at resting tension or relax these rings when pre-contracted.

These results demonstrate that ICI 169,369 causes an endothelium-dependent relaxation of rabbit aorta mediated by EDRF. This relaxation is not elicited by the activation of any known 5-HT, adenosine, histamine or muscarinic receptor or adrenoceptor. Further studies are required to elucidate its mechanism of action.

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FMRFAMIDE POTENTIATION OF 5-HT INDUCED RELAXATION OF THE LOCUST FOREGUT IS NALOXONE SENSITIVE

S.E. Banner & R.H. Osborne, Department of Science, Bristol Polytechnic, Coldharbour Lane, Frenchay, Bristol BS16 1QY

The isolated foregut of the locust Schistocerca gregaria is relaxed in a dose dependent manner by activation of a ketanserin-sensitive 5-HT_2 like receptor (Banner et al., 1987b). The molluscan cardioexcitatory tetrapeptide FMRFamide (Phe-Met-Arg-Phe) (Price & Greenberg, 1977) has been shown to have structural similarities with the enkephalins (Greenberg et al., 1983). Furthermore, the cardiovascular effects of centrally administered FMRFamide and met-enkephalin in rats are blocked by naloxone (Wong et al., 1985) suggesting that both peptides were interacting with mammalian opioid receptors. Recently, Raffa & Bianchi (1986) showed that the contractile effects of acetylcholine on smooth muscle of Mytilus edulis were potentiated by FMRFamide while Walther & Schiebe (1987) showed that a FMRFamide-like peptide modulated neuromuscular transmission in the locust extensor-tibiae preparation. In this report we describe naloxone-sensitive potentiation of 5-HT induced relaxation of the isolated locust foregut by FMRFamide.

Isolated locust foreguts (oesophagus to proventriculus) were incubated in Clarke Insect Ringer at 18 ± 2 °C and tissue tone maintained by alternate standard doses of the pentapeptide proctolin (10^{-7} M) and 5-HT (10^{-6} M) using a six min cycle with two washes. The effect of FMRFamide (2×10^{-5} M) was tested by simultaneous addition with doses of 5-HT. Ketanserin (5×10^{-5} M) and naloxone (10^{-5} M) were used to investigate the action of FMRFamide, the tissue being incubated with an antagonist for 20 min prior to retesting the effects of the agonists. The percentage values are the mean of 8 replicates (± standard error of the mean, S.E.).

FMRFamide had no direct effect on the tissue at the dose used but, in the presence of the tetrapeptide, the relaxation caused by a standard concentration of 5-HT was increased by an average of 36 ± 2.8%. Ketanserin caused a marked reduction (75 ± 5%) in the tissue response to 5-HT and also abolished FMRFamide induced potentiation of the 5-HT effect. By comparison, naloxone had no effect on the response caused by a standard dose of 5-HT but abolished FMRFamide induced potentiation of the relaxant effect of 5-HT.

The data presented above suggest that while FMRFamide had no direct effect on the tissue, it was capable of potentiating 5-HT-induced relaxation by activation of a naloxone-sensitive receptor. It is proposed that FMRFamide exerts its modulatory role by activating a naloxone sensitive receptor which results in an increased response to 5-HT. This concept is supported by the observations that 5-HT relaxation is unaffected by the presence of naloxone and that ketanserin reduces the actions of both the indolealkylamine and FMRFamide. Thus, the effects of both substances are likely to be expressed by activation of the ketanserin-sensitive 5-HT_2 receptor.

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FENFLURAMINE AND DOI DO NOT ACT ON THE SAME 5-HT RECEPTOR TO PRODUCE HYPERTHERMIA IN RATS

J. Pike, E.J. Kidd and C.A. Marsden, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Fenfluramine increases rectal temperature in heat-acclimatized rats (Sulpizio et al, 1978). This effect is mediated centrally (Blackburn, unpublished data) and attenuated by 5-HT_2 receptor antagonists (Blackburn et al., 1984). Recently, Glennon et al (1986) described a new class of 5-HT_2 receptor agonists of which 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) is the most potent. The present study compares the hyperthermic effects of fenfluramine and DOI in heat-acclimatized rats with the aim of determining the 5-HT receptor sub-types involved.

Male, Wistar rats (200-250g) were placed in a room maintained at 26.5 - 28.5°C for 2 hours prior to all experiments. Rectal temperature was measured by inserting a thermistor probe connected to a digital thermometer to a depth of 5 cm into the rectum. Agonists were injected intraperitoneally (i.p.) after a baseline temperature reading and the temperature recorded every 15 min for the next 120 min. In antagonist studies a baseline temperature reading was taken, the antagonist was injected i.p., the agonist given 60 min later and rectal temperature recorded 90 min later. Fenfluramine (2.5 - 20 mg/Kg) produced a dose-related rise in rectal temperature over saline with a maximum increase of 2.33°C occurring 90 min after 20 mg/Kg. DOI (0.1 - 1 mg/Kg) also produced a dose-dependent rise in rectal temperature, with a maximum increase of 1.98°C occurring 120 min (1 mg/Kg). The hyperthermic effects of DOI (1 mg/Kg) were completely antagonised by methiothepin (5-HT_1/5-HT_2 receptor antagonist, 1 mg/Kg) and by mianserin (5-HT_2/5-HT_{1C} receptor antagonist, 0.6 and 2 mg/Kg). Methiothepin and mianserin (2 mg/Kg) alone produced small hypothermic responses but these were not sufficient to account for the blockade of the DOI response. Neither ketanserin (0.2 mg/Kg) nor ritanserin (5 mg/Kg) (5-HT_2 receptor antagonists) altered the hyperthermic response induced by DOI while also having no effect on temperature on their own. In contrast, the hyperthermic response to fenfluramine (15 mg/Kg) was antagonised by both methiothepin (1 mg/Kg) and ketanserin (0.2 mg/Kg).

The results indicate that while DOI and fenfluramine produce hyperthermia in heat-acclimatised rats, the 5-HT receptors involved are different. Fenfluramine-induced hyperthermia may require 5-HT_2 receptors while that induced by DOI appears to involve a 5-HT receptor that is not antagonised by either ketanserin or ritanserin. DOI has been shown to have affinity for 5-HT_{1C} receptors while McKenna et al (1988) have suggested there are two subtypes of the 5-HT_2 receptor; based on differences in the affinity of [^{3}H]-DOI and [^{3}H]-Ketanserin for these sites. The hyperthermia produced by DOI may be a means of functionally distinguishing between these sites.

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DOES NPY RELEASE EDRF IN THE PERFUSED RAT KIDNEY?

D.V. Carter & J. Halliday, Pharmacology Group, Biomedical Science Division, King's College London, Strand, WC2R 2LS.

Neuropeptide Y (NPY) is a tyrosine-rich 36 amino acid peptide shown to be associated with vascular sympathetic nerves and to be present in the bloodstream of man, basal levels being approximately 30pmol/l rising to 90pmol/l under conditions of stress (Lundberg *et al*, 1985). It has already been shown in the rabbit ear artery that the endothelium may have a role in NPY's action in potentiating constrictor responses to sympathetic nerve stimulation (Daly & Hieble, 1987). However, we have shown in the rat kidney that the involvement of the endothelium in NPY potentiation of α-adrenoceptor-mediated responses in blood vessels is not universal (Carter *et al*, 1988) but, in both situations, the concentration of NPY required to elicit potentiation exceeds those found in the circulation. In the present study we have investigated whether the endothelium may play a role in the response to NPY in the rat kidney at more physiological doses than those used to elicit potentiation.

Kidneys were taken from male Wistar rats weighing 200-350g and cannulated via the renal artery and perfused at a constant flow rate of 4ml/min with oxygenated (O_2/5%CO_2) Krebs' solution at 37°C, giving basal perfusion pressures of 50-80 mmHg. Tone was raised by adding 0.5μM phenylephrine to the perfused Krebs. Responses were measured as changes in inflow perfusion pressure.

At doses of 1pmol-0.01pmol NPY a small but significant relaxation was seen in all kidneys studied (n=6) which was less than 30% of that produced by 15nmol carbachol. The relaxation peaked within 15-20 sec. At the higher doses of 1 and 0.1pmol a small contraction often followed the relaxation. None of the above doses of NPY potentiated the response to 15nmol carbachol when administered 30 sec before.

The relaxant phase of both NPY and carbachol was found to be enhanced by 15 Units /ml superoxide dismutase, administered 3 min prior to the bolus injection of NPY, whilst methylene blue (10μM) perfused in the Krebs abolished the relaxant phase.

This evidence suggests that, similar to other peptides such as bradykinin (Toda *et al*, 1987) and endothelin (Warner *et al*, 1988), NPY is able to stimulate the release of EDRF from endothelial cells.

D.V.C. is an MRC scholar.

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PROFILE OF WHR1049 AT α_1- AND α_2 -ADRENOCEPTORS

A.G. Roach, T.L. Berridge, B. Gadie, June Samuel, Patricia Rowland, C.F.C. Smith and I.F. Tulloch, Reckitt & Colman plc, Pharmacology Dept., Dansom Lane, Hull.

In the search for a potent, selective, full α2-adrenoceptor agonist with restricted access to the CNS, we studied the pharmacological profile of WHR1049 (WHR). WHR is the desmethyl metabolite of lidamidine, an antidiarrhoeal agent with weak α2-agonist properties (Dhar et al., 1983), and possesses potent antidiarrheal and antimotility effects in rats (Eaker et al., 1988).

WHR was a full agonist at prejunctional α2-adrenoceptors in the isolated rat vas deferens (RVD) and guinea-pig ileum (GPI) having IC50 values of 13±1.6 and 250±18 nM (n=4-6), respectively. In the RVD, WHR was about 8 times less potent than UK-14,304 (IC50 1.5±0.16 nM) and idazoxan had a pA2 value of 8.15±0.2 against WHR in this tissue. WHR (>300 nM) caused contractions of the isolated rat anococcygeus muscle which were inhibited by prazosin, but not by idazoxan.

The in vivo α1/α2-agonist selectivity of WHR was assessed in the anococcygeus (anoc) muscle of pithed rats as described by Doxey (1979). Both WHR and UK fully inhibited the stimulation-evoked contractions of the anoc; the latter agonist being the more potent compound at both pre α2- and post α1-receptors in the anoc (Table 1). In pithed rats WHR evoked dose-related rises in diastolic blood pressure (DBP; see Table for ED50 value) which were antagonised by the selective α2-antagonist RX811059 (Doxey et al., 1984; 0.3 mg/kg producing a 64-fold parallel dose-ratio shift) and were unaffected by prazosin (1.1 dr shift with 0.1 mg/kg). WHR caused a complete inhibition of stimulation-evoked contractions of the vas deferens in pithed rats, an effect which was antagonised by RX811059 (0.03 mg/kg producing a 7.3-fold dr shift). The agonist potencies of WHR and UK in the various test systems in pithed rats are shown in Table 1 along with the calculated α1/α2-potency ratios.

	Agonist Potencies (μg/kg)				Potency Ratios		
	Post α2 DBP ED50	Pre α2 Vas ED50	Pre α2 Anoc. ED50	Post α1 Anoc. ED1.0g	Post α1 Post α2 (DBP)	Post α1 Pre α2 (Vas)	Post α1 Pre α2 (Anoc.)
WHR1049	8.3	2.8	16.6	2617	315	934	158
UK-14,304	6.7	2.3	4.2	1705	254	741	405

Table 1: α1/α2-Adrenoceptor potencies and potency ratios of WHR1049 and UK-14,304 in pithed rats. ED50 values denote doses to increase DBP by 50 mmHg or produce 50% inhibition of stimulation-evoked contractions of vas and anoc. ED1.0g is the dose increasing resting tension of anoc. by 1.0g. All values are means±sem of groups of at least six rats.

At central α2-receptors both UK and WHR evoked full mydriasis in pentobarbitone-anaesthetised rats (ED50 values for UK and WHR, doses causing 50% pupil dilatation, were 50±7 and 443±10.7 μg/kg, i.v.). The responses to UK were rapid in onset (2-3 min) whereas those to WHR were slow (30 min) to develop. Thus, WHR is a potent, selective, full α2-adrenoceptor agonist which penetrates the CNS of rats less readily than UK.

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THE PORTAL HYPOTENSIVE EFFECT OF β-ADRENOCEPTOR AGONISTS IN THE RAT

E Burns, TM Broadhead, NDS Bax University department of Pharmacology and Therapeutics, Royal Hallamshire Hospital, Glossop Road, Sheffield

Beta-adrenoceptors are present in the portal vein in animals and man and mediate vasodilatation. However, in man, propranolol, a beta-adrenoceptor antagonist has been used to treat portal hypertension secondary to hepatic cirrhosis, and although not always effective, may mediate its effect by decreasing cardiac output and thus portal blood flow. In a preliminary study we have demonstrated that the beta-adrenoceptor agonists salbutamol (S) and isoprenaline (I) lower portal venous pressure (Ppv) in an isolated perfused rat liver (IPRL) primed with a noradrenaline (NA) bolus (Burns et al, 1988). In the present experiment the effcts of I,S and propranolol (P) on the Ppv in the IPRL were examined, but on this occasion using a NA infusion (200ng/min) to mimic the NA concentrations in cirrhotic patients (Ring-Larsen, 1982).

The livers of 39 male Wistar rats (mean±sd body weight 233±19g) were perfused in situ (perfusate volume 100ml, flow rate 10 ml/min) (Burns et al, 1989, in press). The Ppv and hepatic venous pressures (Phv) were monitored continuously. After a 30 min stabilisation period the NA infusion began and 30 min later the test drug was added as a bolus to the perfusate. The test drug concentrations and the experimental results are shown in the Table. The results are given as mean values (sd).

Drug	Conc. I,S or P uM	n	Pretreatment Ppv (cm of water)	% increase in Ppv after NA	Change in Ppv at times after I, S or P (Δ % of Ppv after NA) 4 min	10 min
NA		7	9.1 (2.3)	31 (12)	+0.1 (1.7)	+0.3 (1.0)
NA+I	100	6	9.9 (1.3)	20 (9)	-1.9 (4.1)*	-2.5 (2.2)**
NA+I	10	6	9.3 (1.2)	25 (10)	-7.6 (3.6)**	-7.9 (2.9)**
NA+I	1	6	9.0 (2.1)	23 (11)	-7.3 (1.9)**	-6.6 (1.7)**
NA+S	10	8	9.4 (1.7)	29 (6)	-7.1 (3.0)**	-5.2 (2.3)**
NA+S	1	6	9.0 (1.3)	26 (8)	-7.4 (3.4)**	-6.9 (2.9)**
NA+P	100	4	9.0 (0.7)	32 (4)	+1.5 (1.4)	+1.2 (0.9)

*$p<0.01$, **$p<0.001$ for differences between NA alone and NA+I, S or P.

Analysis of variance showed no significant differences between the pretreatment Ppv values or between the percentage rise in pressure after the NA infusion. None of the drugs produced any significant alteration in Phv. At concentrations below 100uM P produced no significant effect on Ppv.

Thus the addition of beta-adrenoceptors agonists to the IPRL caused a fall in the elevated Ppv produced by the infusion of NA. The portal hypotensive action of salbutamol suggests that this phenomenon may be mediated by beta-2 receptors. The lack of effect of P on the Ppv suggests any portal hypotensive effect it causes in man results from its effect on cardiac output. Salbutamol warrants further investigation as a possible treatment of portal hypertension in man.

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LACK OF DOWN REGULATION OR DESENSITISATION OF RABBIT AORTIC α_1 ADRENOCEPTORS AFTER LONG TERM IN VIVO NORADRENALINE INFUSION

J.J Boyle, C.A. Hamilton & J.L. Reid, Department of Materia Medica, Stobhill General Hospital, Glasgow, G21 3UW.

Agonist induced down regulation and desensitisation is well documented and has been demonstrated for vascular smooth muscle alpha$_1$ adrenoceptors in culture and in vitro (Wikberg et al 1983; Lurie et al 1985). We have previously shown down regulation of alpha$_2$ and beta adrenoceptors during 10 day in vivo noradrenaline infusions (Deighton et al 1988). We have now studied the effects of long-term noradrenaline infusion on vascular alpha$_1$ adrenoceptors in rabbit aortae. Animals were infused with noradrenaline (0.09 um/kg/hr) as previously described using Alzet osmotic mini pumps. At the end of the infusions animals were killed, the aortae removed, washed 4 x 10 min in Krebs Ringer Buffer at 37^{o}C and cut into 1 mm rings. Membranes were prepared from some rings and used for measurement of alpha receptor number and affinity using the alpha$_1$-adrenoceptor selective ligand [^{125}I] HEAT (Jones et al 1987). Other rings were used to examine phosphotidyl inositol hydrolysis. 8 x 1 mm rings were incubated in the presence of lithium chloride 10^{-2}M and 1.5 uCi [^{3}H] inositol for 4 hr with additon of noradrenaline or vehicle after 3 hr. Inositol phosphates were extracted into $CHCl_3$: MeOH: HCl and isolated by anion exchange chromatography (Fox et al 1985).

Plasma noradrenaline was significantly increased in treated animals, 17 $\pm$ 4 nM compared to 3 $\pm$ 1 nM in control animals. 10 days exposure to these noradrenaline concentrations failed to alter [^{125}I] HEAT binding in aortae, B_{max} being 73 $\pm$ 17 and 83 $\pm$ 11 fmoles/mg protein and K_D 58 $\pm$ 12 and 84 $\pm$ 14 in control and treated animals (mean $\pm$ SE n = 6). Similarly, neither basal nor noradrenaline stimulated phosphotidyl inositol hydrolysis was altered by noradrenaline infusion. Noradrenaline 2 x 10^{-6}, 10^{-5} and 10^{-4} M caused increases in inositol phosphates to 170 $\pm$ 10, 230 $\pm$ 40 and 290 $\pm$ 40% of basal levels in treated animals and 160 $\pm$ 20, 240 $\pm$ 30 and 290 $\pm$ 40% in controls (n = 7-8). Thus 10 day infusions with moderate doses of noradrenaline which can down regulate alpha$_2$ and beta adrenoceptors did not alter either alpha$_1$ adrenoceptor number as measured by [^{125}I] HEAT or phosphatidyl inositol hydrolysis in rabbit aorta. In previous in vitro studies higher doses of noradrenaline (10 - 100 uM) have generally been used and the lack of agonist down regulation in this in vivo study may be related to the relatively low doses of noradrenaline to which the alpha$_1$ adrenoceptors were exposed.

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WHICH α-ADRENOCEPTOR SUBTYPE DOES PHENYLEPHRINE STIMULATE TO CAUSE CONTRACTION IN DOG SAPHENOUS VEIN IN VITRO?

M. Barras, G. Herman[1], P. Mauduit[1], J.M. Armstrong, B. Rossignol[1] & P.E. Hicks* Department of Pharmacology, Recherche Syntex France, 91310 Montlhéry and [1]Institut de Biochimie, Université de Paris Sud, 91405 Orsay, France.

Phenylephrine (PHE) has been considered a selective agonist at alpha$_1$-receptors, however, in dog saphenous vein (DSV) the specificity of this agonist is debatable. Thus prazosin (PRZ) showed low antagonist potency on PHE-induced contractions (Alabaster et al, 1985) and the alpha$_2$-antagonist yohimbine (YOH) higher potency than normally associated with alpha$_1$-receptors (Sullivan & Drew, 1980). Furthermore, YOH showed similar antagonist effects on PHE or UK-14304 (alpha$_2$) -induced contractions (Guimaraes et al, 1987) implying that PHE can stimulate alpha$_2$-receptors in DSV. PHE can however, increase the formation of inositol phosphates (IPs) in DSV, a secondary messenger system not associated with alpha$_2$ -receptor coupling and these effects were blocked by PRZ but not by rauwolscine (Rees & Matthews, 1986).

In the present experiments rings of DSV were denuded of endothelium by gentle rubbing, mounted under 2g tension in Kreb's solution containing propranolol (1uM) and bubbled with carbogen. Contractile-concentration curves were induced by PHE or BHT-920 as agonists in separate groups (n=4-7), in normal or $[Ca^2]_0$ Krebs and antagonist affinities (K_B) calculated. In other experiments DSV rings were incubated for 3h in Kreb's containing myo-^{3}H-inositol (60 uCi/ml), then washed with 10 mM inositol and placed 4-5/tube for 10 min in Krebs containing LiCl(10 mM) Rings were exposed to antagonists for 30 min prior to addition of PHE (1-1000 uM) for 10 min, immersed in liquid nitrogen, lyophilised for 18h, homogenised in 7.5% w/v TCA, centrifuged and TCA extracted with ether. [^{3}H]-IP$_1$, IP$_2$ and IP$_3$ were eluted stepwise on ion exchange columns (Berridge et al, 1983). Tissue radioactivity was determined by liquid scintillation. Formation of IPs were calculated in cpm/mg dry wt or as % total tissue [^{3}H] inositol.

YOH was a more potent antagonist of BHT (K_B 8.7 ± 0.1; slope 0.7) than PHE (K_B 7.8 ± 0.1; slope 0.7) induced contractions but the antagonism was not competitive. Against PHE, corynanthine (CORY; K_B 7.3 ± 0.2) was more potent than idazoxan (IDZ; K_B 6.3 ± 0.2) but was less potent (K_B 6.3 ± 0.1) than IDZ (K_B 7.5±0.1) against BHT. The potency of YOH against PHE did not increase in the presence of 0.3uM PRZ (K_B 7.5 ± 0.1). In $[Ca^2]_0$ solution, PHE contracted DSV to 60% of a control KCl (80mM) response and these effects were blocked by YOH (K_B 8.2 ± 0.3; n=5). Stimulation with PHE (100 uM; 10 min), increased total IPs comprising IP$_1$(77%), IP$_2$(20%) and IP$_3$(3%). The response-curve relating [PHE] to total IP formation was shifted to the right of controls by YOH (0.1uM), but YOH (0.1uM) + PRZ (0.1uM) showed greater inhibitory effects only on the maximum PI-response. IDZ (0.3 uM) had little effect on PHE (100 uM)-stimulated IP formation.

Thus antagonist affinity values for YOH against PHE-induced contraction were not modified in $[Ca^2]_0$ or in the presence of PRZ, but were lower than against BHT. YOH but not IDZ blocked PHE-stimulated IP formation. Taken together these results suggest that PHE-induced contractions in DSV are mediated by an alpha$_1$'like'-receptor at which YOH, CORY and PRZ have equivalent antagonist affinities, IDZ low affinity and which utilize the PI-response as a secondary messenger system. These data provide little evidence that PHE stimulates postsynaptic alpha$_2$-receptors in DSV (Guimaraes et al, 1987) as defined using agonists like BHT-920.

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THE EFFECTS OF GLIBENCLAMIDE ON THE ACTIONS OF CROMAKALIM IN GUINEA-PIG PORTAL VEIN

A.D. Wickenden, S. Grimwood & T.L. Grant. (Introduced by N.J.W. Russell). ICI Pharmaceuticals, Bioscience Dept II, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

It has been reported that the inhibition of spontaneous mechanical activity of the rat and guinea-pig portal vein by the K^+ channel opener cromakalim (CROM), in contrast to the anti-spasmogenic activity of CROM in these tissues, is not associated with an increase in $^{86}Rb^+$ efflux (Shetty and Weiss, 1987 and Quast, 1987). It has been suggested that this discrepancy merely reflects the inability of the assays employed to detect small increases in efflux associated with K^+ channels located on pacemaker cells. However, the possibility remains that inhibition of spontaneous activity and spasmogenic activity by CROM are mediated by different mechanisms. In order to investigate this, the present study examines the effect of the K^+ channel blocker Glibenclamide (GLIB) against the inhibitory action of CROM on spontaneous activity and KCl induced contractions in the guinea-pig portal vein.

Longitudinal or circular sections of guinea-pig portal vein were suspended in Kreb's solution at 37°C and gassed with 95%O_2/5%CO_2. Changes in isometric tension were recorded under a resting tension of 1.0g and 0.5g respectively. Inhibition of spontaneous activity by CROM was assessed cumulatively in longitudinal sections before and after the addition of GLIB (0.1–10μM, 30 mins incubation) or vehicle. Inhibition of KCl induced contractions (10–70mM) by 10μM CROM was assessed in circular sections of portal vein in the presence of GLIB (1–10μM) or vehicle.

CROM inhibited spontaneous mechanical activity in the guinea-pig portal vein with a mean IC_{50} (± S.E.M.) 0.02±0.003μM. GLIB (0.1, 1 and 10μM) produced a dose related rightward shift of the CROM dose response curve (IC_{50} 0.2±0.09, 1.92±0.8 and 7.45±1μM respectively). In addition the inhibition was apparently competitive in that no depression in maximum inhibition was observed. Schild analysis of these data revealed a linear relationship with slope not significantly different to unity and a pA_2 of 7.5 (8.1–6.9). Similarly the inhibitory effects of CROM (10μM) against KCl induced contractions is inhibited in dose related manner by GLIB over a similar concentration range to the extent that 10μM CROM was without effect in the presence of GLIB (10μM).

In conclusion, it appears that inhibition of spontaneous activity and KCl induced contraction by CROM are mediated by a common GLIB sensitive mechanism and that the reason for the failure to detect $^{86}Rb^+$ efflux in the presence of CROM concentrations that inhibit spontaneous activity reflects a lack of sensitivity of the $^{86}Rb^+$ efflux technique.

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SIMILARITIES BETWEEN THE CONTRACTILE EFFECTS OF NEUROPEPTIDE Y AND Bay K8644 IN RABBIT ISOLATED ARTERIES

Anne Verity and S E O'Connor, Department of Pharmacology, Fisons plc - Pharmaceutical Division, Bakewell Road, Loughborough, LE11 ORH.

Neuropeptide Y (NPY) demonstrates a direct vasoconstrictor action in vitro which varies considerably in prominence between different tissues. For example, in rabbit basilar artery and guinea pig iliac vein NPY contracts with high intrinsic activity, whereas many peripheral arteries are virtually unresponsive (Edvinsson et al, 1987, Thorpe et al, 1987). The basis of this tissue variation is not clear. Contractile effects of the calcium channel activator BAY K8644 also vary, being markedly more pronounced in quiescent cerebral than peripheral arteries of the dog (Asano et al, 1987). We have compared these two agents directly to investigate the extent of their similarity. Noradrenaline was also included in the study.

Male NZ white rabbits (2.5-3.5 kg) were killed with an overdose of pentobarbitone and basilar, femoral and gastroepiploic arteries removed. Ring sections from each vessel were set up in organ baths in Krebs buffer (5.8 mM KCl) at 37°C, bubbled with 95% O_2/5% CO_2 under resting tensions of 0.5 g or 1 g (femoral only). After a 1 h equilibration period tissues were exposed first to 40 mM KCl, and then to 80 mM KCl to define the tissue maximum. A single cumulative concentration response curve (CRC) was then constructed to either BAY K8644 (10^{-10}-$3x10^{-6}$M), NPY (10^{-9}-$3x10^{-7}$M) or noradrenaline ($3x10^{-10}$-10^{-5}M). In some tissues full curves could not be established, therefore, EC_{50} values are not shown. Contractions were expressed as a percentage of the response to 80 mM KCl. Maximum responses obtained are shown in Table 1.

Table 1

	n	Maximum response obtained (% KCl, mean ± s.e.) Basilar	Femoral	Gastroepiploic
BAY K8644	5-15	91 ± 11%	15 ± 6%	6 ± 2%
NPY	5-17	54 ± 4%	11 ± 4%	5 ± 2%
Noradrenaline	3-7	42 ± 4%	131 ± 12%	126 ± 7%

NPY and BAY K8644 were alike in that both contracted the basilar artery strongly but demonstrated only weak effects in femoral and gastroepiploic arteries. Noradrenaline showed the opposite profile. To investigate these differences further, CRC's to NPY and BAY K8644 were repeated in the poorly responsive arteries in the presence of 15 mM KCl (just threshold for contraction). This resulted in a significant augmentation of the effects of both agents with increased maximum response (femoral - NPY 33 ± 4%, BAY K8644 66 ± 7%, gastroepiploic - NPY 25 ± 5%, BAY K8644 54 ± 9%, t-test $P<0.05$ v max in normal Krebs).

The dependence, in most tissues, of BAY K8644-induced contractile activity on partial membrane depolarisation (i.e. 15 mM KCl) is well known (Schramm et al, 1983). This characteristic was shared by NPY in our study. The observed similarities between NPY and BAY K8644 suggest that NPY opens voltage operated calcium channels (VOC), possibly as a result of membrane depolarisation, as proposed recently to account for other actions of NPY (Andriantsitohaina and Stoclet, 1988). Differences in resting membrane potential between vessels and, therefore, the likelihood of VOC activation, may explain the variation observed in the direct constrictor effects of NPY.

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THE EFFECT OF AMMONIUM CHLORIDE ON MEMBRANE POTENTIAL AND TONE OF ISOLATED MESENTERIC ARTERIOLES

A.D. Hughes. C. Aalkjaer. M. Mulvany. Institute of Biophysics, Aarhus University. Aarhus, Denmark.

Changes in intracellular pH may affect a number of processes involved in excitation contraction coupling, including myofilament calcium sensitivity (Fabiato & Fabiato 1978), calcium currents (Irisawa & Sato 1986), phosphoinositide metabolism (Griendling et al., 1987) and membrane potential (Em) (Harder 1982). Exposure of tissues to ammonium chloride (NH4) is a well established technique used to induce changes in intracellular pH (Thomas 1984). In vascular smooth muscle NH4 induces a transient alkalinisation followed by a more prolonged acidification after washout. We have therefore investigated the effect of NH4 on Em and tone in resting or noradrenaline activated resistance arteries.

Segments of resistance arteries (ca. 1.5mm long, internal diameter ca. 200μm) were taken from the mesenteric bed of normotensive Wistar Kyoto rats and mounted in a myograph containing physiological saline (PSS) (37°C, 95% O_2, 5% CO_2), bicarbonate containing (HCO_3-HEPES) or bicarbonate free HEPES buffer (HEPES) (37°C, 100% O_2) to allow measurement of isometric tension. Vessels were set to an internal circumference Lo = 0.9 L100, where L100 is an estimate of the internal circumference the vessel would possess when distended by 100mm Hg transmural pressure (Mulvany & Halpern 1977). Solutions were continuously circulated through the myograph chamber by means of a peristaltic pump. Intracellular recordings of Em were made using glass microelectrodes (30-100MΩ) which allowed continuous monitoring of both Em and tension.

Em in PSS was 56± 1mV (mean± s.e.mean, n = 29), 56± 2mV (n = 5) in HCO_3-HEPES (NS.) and 52± 1mV (n = 19) in HEPES ($P<0.01$). Exposure of tissues to NH4 (10mM) for 10 mins and subsequent washout had no significant effect on Em or tone of resting arteries in either PSS or HEPES. In vessels contracted by noradrenaline (2μM) addition of NH4 caused a marked relaxation with a transient increase in tone following washout of NH4. This procedure was not associated with any significant alteration in Em in either PSS or HEPES (n = 8).

In conclusion, although NH4 has effects on the tone of resistance arteries contracted by noradrenaline it seems unlikely that this action is due to an effect of NH4 on Em.

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PLATELET ACTIVATING FACTOR AND ENDOTOXIN-INDUCED DEPRESSION OF VASCULAR REACTIVITY

Gillian A. Gray, B.L. Furman and J.R. Parratt, Department of Physiology and Pharmacology, Strathclyde University, Glasgow G1 1XW, Scotland

Previous work showed that endotoxin, independently of its hypotensive action, depresses vascular responsiveness to noradrenaline (NA) and angiotensin, (Gray et al. 1987). The mechanism of this effect is unknown, although it was prevented by cyclooxygenase inhibitors. Endotoxin was reported to increase the production of platelet activating factor (PAF) (Doebber et al. 1985). As PAF is a potent vasoactive substance we have examined its possible role in mediating the impaired vascular responsiveness induced by endotoxin. Rats were pithed under ether and ventilated artificially (100% O_2; 53 strokes/min, approximately 1ml/100g to keep arterial PCO_2 at about 30 mm Hg). Rectal temperature was maintained at 37 ± 0.5 °C. Cannulae were placed in the carotid arteries for blood sampling and for measurement of arterial blood pressure, and in the femoral vein for drug administration. The pithing rod was positioned for electrical stimulation (ES) (50 V; 0.1 msec for 5 sec) of the sympathetic outflow at T8 (Gillespie et al. 1970). All rats were infused with gallamine (10 mg kg^{-1} h^{-1} after a 10 mg/kg bolus). After 1h equilibration, control responses were obtained and E. coli endotoxin (Difco O55:B5, 1.0 mg/kg) or 0.9% saline were infused over 1h. Infusion of endotoxin produced a fall in mean arterial blood pressure which was maximal at 60 min (control 55 ± 2 mm Hg; endotoxin 42 ± 4 mm Hg; $P<0.05$). Pressor responses to NA (100 ng/kg) were constant over 1h in saline-infused rats but were depressed in endotoxin treated rats (e.g. pressor response to NA at 60 min in control rats was 24 ± 3 vs 9 ± 1 mm Hg in endotoxin-treated rats; $P<0.05$ n = 6-8). Similarly, the response to ES (1-10 Hz) was reduced (e.g. pressor response to ES at 5 Hz in control rats was 38 ± 5 mm Hg vs 19 ± 6 mm Hg in endotoxin-treated rats $p< 0.05$). PAF (250 ng/kg) produced an immediate and long-lasting hypotension (control pressure 63 ± 5 mm Hg, 42 ± 3 mmHg 30 min after PAF; $P<0.05$). Responses to NA were depressed at 30 min but not at 60 min after PAF, although mean arterial blood pressure remained low (control response to NA was 19 ± 2 vs 12 ± 3 mm Hg at 30 min after PAF; $P<0.05$). The response to ES (1-10 Hz) remained depressed at 60 min after PAF (e.g. control response to 5 Hz was 38 ± 5 mm Hg vs 23 ± 6 mm Hg after PAF; $P<0.05$). Administration of the PAF antagonist, WEB 2086 (5mg/kg, 10 min before endotoxin) delayed endotoxin-induced hypotension but did not modify the depressed vascular reactivity. In this dose WEB 2086 completely prevented the vasodepressor response to PAF. Although PAF may contribute to endotoxin-induced hypotension, its role in the impairment of vascular reactivity remains unclear.

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ALTERED RESPONSIVENESS IN THE ISOLATED PERFUSED MESENTERIC VASCULAR BED OF THE DIETARY OBESE RAT

FELICITY S.M. LEIGH, MARILYN J. KIRBY, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY

The isolated perfused mesenteric bed has been shown to be of use in studying the responsiveness of blood vessels to noradrenaline in hypertension (DOCA salt type), demonstrating an increased sensitivity in tissues from hypertensive rats (Longhurst et al, 1988). Increases in maximal responses to noradrenaline in the mesenteric vasculature from dietary obese rats (which show elevated blood pressure compared with lean controls) have also been reported (Kirby & Leigh, 1986). The current study extends this latter work to include the response to other agents.

Female Wistar rats, University of Bath strain, of initial weight 203.9±4.4g (mean±s.e.m) were housed in cages of 6 or 8 rats under conditions of 12:12 hours light:dark, at 22°C. All rats received chow and water throughout the study and, in addition, the dietary obese rats received an excess of 4 palatable foods daily. Animals were killed after 4,8,10, and 15 weeks of study and the isolated perfused mesenteric bed studied using the methods of McGregor (1965) (contractile responses) and Byfield et al (1986) (relaxation). Responses to the following substances were investigated (i) bolus injections of 5-hydroxytryptamine (5-HT) (contraction) after 4 and 15 weeks (ii) bolus injections of acetylcholine (ACh) (relaxation, after contraction with Krebs solution containing 10^{-3}M and 10^{-4}M phenylephrine) after 4 and 10 weeks (iii) perfusion with Krebs solution containing either 65mM potassium (K^+) or 65mM K^+ plus calcium (Ca^{2+}) (contraction), after 4 and 8 weeks.

Maximal responses to 5-HT (increase in perfusion pressure, mmHg) were greater for tissue from obese rats than controls after 4 weeks (61.6±7.5 and 35.2±4.1, both n=8, $p<0.001$) and after 15 weeks (66.9±6.5, n=11 and 34.6±6.1, n=10, $p<0.005$). ED_{50} of 5-HT for tissues from obese rats (32.4±2.9 x 10^{-10}mole) was lower than for lean (44.4±5.6 x 10^{-10}mole) after 4 weeks, $p<0.005$. ED_{50} of ACh for tissues from obese rats was greater than for lean after 4 weeks (11.7±3.3 x 10^{-11}, n=8, and 5.95±0.86 x 10^{-11}mole, n=6, $p<0.05$).

65mM K^+ and 65mM K^+/3mM Ca^{2+} Krebs solutions produced similar contractile responses in tissues from lean and obese rats after 4 weeks. After 8 weeks, the plateau pressure (mmHg) achieved with 65mM K^+ Krebs solution was greater for obese (55.0±4.5, n=10) than for lean (42.6±1.5, n=8), $p<0.001$ and the plateau pressure achieved with 65mM K^+/3mM Ca^{2+} Krebs solution was also greater for obese (71.3±7.0, n=10) than for lean (55.9±2.9, n=8), $p<0.005$. Time to achieve plateau pressure with "normal" Krebs solution (minimum pressure) was greater for tissues from obese than lean rats (17.04±0.88 and 12.46±0.89 minutes), $p<0.001$. Time to plateau pressure with 65mM K^+ solution (minimum pressure) was also greater for tissues from obese than lean rats (16.27±1.53 and 10.25±1.09 minutes), $p<0.001$. However, time to plateau pressure with 65mM K^+/3mM Ca^{2+} Krebs solution (maximum pressure) was less for tissues from obese than from lean (21.82 ±1.92 and 27.00±0.95 minutes), $p<0.001$.

Thus it can be seen that dietary obesity in the rat not only results in elevated blood pressure but also causes changes in the mesenteric bed, as illustrated by the responses of the isolated tissue to ACh, 5-HT and concentrations of potassium and calcium in the perfusing Krebs solution.

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VARIATIONS IN THE DISTRIBUTION OF [^{125}I]-ENDOTHELIN BINDING SITES WITHIN HUMAN VASCULAR TISSUE: AN AUTORADIOGRAPHIC STUDY

M.R. Dashwood[1,2], R. Sykes[2], A.H. Chester[1] and M. Yacoub[1], [1]Harefield Hospital, Uxbridge, Middlesex, [2]Dept. of Physiology, Royal Free Hospital School of Medicine Rowland Hill Street, London NW3 2PF.

Endothelin is a vasoactive peptide with potent constrictor activity in both arterial and venous tissue (Tippins et al 1989). We have described the distribution of [^{125}I] endothelin binding sites as well as the effects of endothelin in vitro, in both the human coronary artery (Chester et al 1989) and rabbit aorta (Dashwood et al 1989). In the present study we have used in vitro autoradiography to localise [^{125}I] endothelin binding sites in human venous and arterial tissue.

Tissues were obtained from patients undergoing heart transplant or cardiac surgery (saphenous vein, internal mammary artery, coronary arteries and carotid artery). 20μm sections were cut in a cryostat and thaw-mounted onto gelatinised microscope slides before being incubated in 50 pM [^{125}I] endothelin (specific activity 1060 Ci/mmol, Peninsula Labs) as described previously (Dashwood et al 1989). The degree of binding to non-specific sites was established by incubating alternate sections in the presence of 500 nM unlabelled endothelin (Peninsula Labs.). Both low (Hyperfilm ^{3}H) and high (K2 emulsion, Ilford) resolution autoradiography was performed. There was a displaceable binding of [^{125}I] endothelin to all vessels studied which varied both in density and distribution. High resolution studies revealed a dense binding in human coronary arteries which was associated with the outer tunica media, as well as to perivascular nerves. There was dense binding to the saphenous vein and a weaker binding to the carotid artery (Fig. 1).

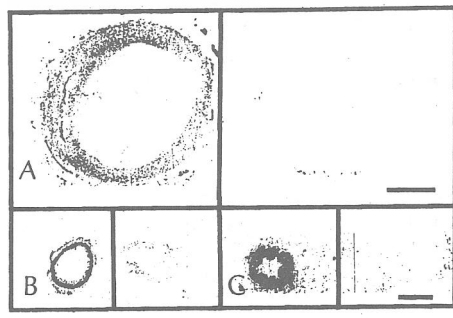

Figure 1: [^{125}I] Endothelin Binding to Human Blood Vessels

A. Carotid Artery
B. Left anterior descending coronary artery
C. Saphenous vein
LEFT - Autoradiographs of total binding
RIGHT - Non-specific binding

Bar in A = 2mm
Bar in B & C = 1mm

There was an increase in the degree of [^{125}I] endothelin binding associated with the tunica media of coronary arteries in the presence of atheroma. Previous functional studies have shown that the constrictor effects of endothelin varies in different vessels (Tippins et al 1989).

These authoradiographic results indicate that the variations in sensitivity may well be due to the difference in receptor density and distribution within the vessels studied.

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BLOOD PRESSURE CHANGES INDUCED BY ENDOTHELIN AND [ALA^{1,3,11,15}]-ENDOTHELIN IN PITHED RATS: EFFECT OF LOWERING VENTILATION VOLUME

Margaret R. MacLean and C. Robin Hiley, Department of Pharmacology, University of Cambridge, Hills Road, Cambridge CB2 2QD.

Hypoxic conditions potentiate arterial vasoconstrictor responses to noradrenaline (Detar & Gellai, 1971) and this is dependent on the presence of the vascular endothelium (De Mey & Vanhoutte, 1982). Endothelium-dependent vasoconstriction also occurrs in response to hypoxia (Rubanyi & Vanhoutte, 1985). Since endothelin (the originally described porcine/human form now being designated ET-1) is a potent vasoactive agent secreted by endothelial cells in culture (Yanagisawa et al., 1988), it is of interest to determine its effects in hypoxia. Here we describe the effects of lowering ventilation volume in pithed rats, on the blood pressure responses to ET-1 and an analogue, [Ala^{1,3,11,15}]ET-1.

Male Wistar rats (250-300 g; Bantin & Kingman) were pithed under halothane anaesthesia and respired with air through a tracheal cannula at 54 cycles/min at a volume of either 20 (control) or 10 ml/kg. The right carotid artery and left jugular vein were cannulated for recording mean arterial blood pressure (MABP) and drug administration, respectively. Drugs were given, in saline, as bolus injections (0.1 - 0.5 ml) and blood pressure allowed to return to baseline before administration of a subsequent dose.

In rats respired at 20 ml/kg (pH=7.47±0.5, PO_2=78.9±2.24, PCO_2=38.3±1.5, n = 5) [Ala^{1,3,11,15}]ET-1 (0.1 - 5 μg) induced dose-dependent, biphasic changes in MABP. At lower doses (0.1 - 2 μg), a prolonged (30 min - 1 h) pressor response was preceded by a transient (30 s) depressor phase. At higher doses only pressor responses occurred. These were also biphasic and comprised an initial, transient (1 min) response, appearing first as a 'shoulder' to the response then as a seperate 'peak' at the highest doses, followed by a maintained pressor response (30 min - 1 h). The maximum depressor response (12 mmHg) occurred at 1.5 μg. The maximum sustained pressor response was 58 mmHg at the 4 μg dose. The ED50 for this sustained response was 1.34 ± 0.16 μg (n = 5). In the same rats, at a respiratory volume of 10 ml/kg (pH=7.2±0.2, PO_2=58.7±2.5, PCO_2 62.2±1.1), [Ala^{1,3,11,15}]ET-1 (synthesized by J.T. Pelton, Merrell Dow, Strasbourg) only gave pressor responses (maximum increase; 43 mmHg with 2 μg). The pressor responses to 0.1 - 1 μg doses were greater than the controls, e.g. the response to 500 ng was 9.2 ± 2.4 mmHg (n = 5) at 20ml/kg respiratory volume and 18.3 ± 4.4 mmHg at 10 ml/kg. In control animals, ET-1 (0.025 - 1 μg; Novabiochem) induced biphasic MABP changes at all doses; an initial, transient (30 s) depressor phase was followed by a sustained (30 min - 1 h) pressor response. 750 ng gave the maximum depressor response of 18 mmHg whilst 500 ng gave the maximum pressor response (90 mmHg). The ED50 for pressor responses to ET-1 was 0.27±0.04 μg (n = 5). At 10 ml/kg respiratory volume, 10 ng ET-1, which had no effect at 20 ml/kg, induced a biphasic response; a depressor response of 3±1.2 mmHg (30 s) and a secondary, prolonged pressor response of 5±0 mmHg (n = 5). Subsequent administration of 30 ng ET-1 induced a depressor response of -15±5 mmHg (n = 3), following which the MABP and heart rate oscillated and decreased until the rat died 10-15 min after ET-1 administration. Hence a dose-response curve for ET-1 in the hypoxic rats could not be constructed.

This study shows that, in the pithed rat, ET-1 is a five times more potent pressor agent than [Ala^{1,3,11,15}]ET-1. Hypoxia, accompanied by hypercapnia and acidosis, potentiated the pressor responses to lower doses of the ET-1 analogue, whilst enhancing both the depressor and pressor responses to ET-1, rendering it toxic to the cardiovascular system at very low doses.

We wish to thank the Merrell Dow Research Institute, Strasbourg, France for the generous gift of the peptides.

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ENDOTHELIN STIMULATES PHOSPHATIDYL INOSITOL HYDROLYSIS IN RAT AORTA

Y-T. Huang, C.A. Hamilton & J.L. Reid, Department of Materia Medica, Stobhill General Hospital, Glasgow, G21 3UW.

Endothelin (E) is a potent vasoconstrictor peptide recently isolated from cultured endothelial cells (Yanagisawa et al 1988). It has been suggested that vasoconstriction is dependent on an influx of extracellular calcium via calcium channels (Yanagisawa et al 1988) although mobilisation of calcium from intracellular stores has also been proposed (Miasiro et al 1988). To test the latter hypothesis we have examined the ability of E to stimulate phosphatidyl inositol hydrolysis (PIH) in rat aorta. 2 x 0.5 cm rings of rat aorta were incubated in Krebs Ringer Buffer with 1.5 uCi [^{3}H] inositol and 10^{-2}M lithium chloride for 4 hr with the addition of agonists or vehicle after 3½ hr unless otherwise stated. Inositol phosphates were extracted into $CHCl_3$:MeOH:HCl and isolated by anion exchange chromatography (Berridge et al 1982).

E caused a dose related increase in PIH (Table 1), the maximum increase being much greater than that produced by noradrenaline (Nor) 10^{-4}M.

Table 1. Agonist induced phosphotidyl inositol hydrolysis.

Agonist (M)	Nor10^{-4}	E10^{-9}	E10^{-8}	E10^{-7}	E10^{-6}	E10^{-5}
% of basal PIH	300 ± 32	125 ± 14	220 ± 25	470 ± 79	740 ± 120	1040 ± 177

Results mean ± SE n = 5-6

PIH was not dependent on the presence of endothelium, but was attenuated when endothelium was removed by rubbing. In the presence and absence of endothelium responses to E10^{-6}M were 990 ± 82% and 430 ± 85% of basal levels. Tachyphalaxis to E has been noted (Auguet et al 1988) and although PIH was initially rapid it decreased with time. After 5, 15, 30 and 60 min PIH was 370, 1000, 1200 and 1110% of basal levels with E10^{-6}M and 160, 300, 590 and 560 with E10^{-7}M. The attenuation of response was not due to substrate exhaustion nor was it attenuated by addition of the protein kinase C inhibitor H_7 (50 uM) 10 min prior to E.

These studies show that E10^{-8} - 10^{-5}M can stimulate PIH in rat aorta. However, in vitro the EC_{50} for vasoconstriction of rat aortic rings is 10^{-9} - 10^{-8} (Auguet et al 1988) while in cultured rat smooth muscle aorta [^{125}I] labelled E binds with an apparent K_D of 10^{-10} - 10^{-9}M and stimulates increases in $[Ca]_i^{2+}$ in the range 10^{-9} - 10^{-7}M (Hirata et al 1988). Thus PIH as measured in the present study does not correlate directly with functional responses.

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MICROCIRCULATORY EFFECTS OF ENDOTHELIN IN THE HAMSTER CHEEK POUCH

B. Rosen & W. Paffhausen (introduced by P.J. Gardiner). Institute for Pharmacology, Bayer AG, P.O. Box 10 17 09, D-5600 Wuppertal, FRG.

Endothelin has been described as a potent vasoconstrictor peptide (Yanagisawa et al.,1988). The aim of this study was to investigate the effects of endothelin on microvessels of different size in the hamster cheek pouch. The pouch of pentobarbital aneasthetized animals was prepared for microscopical observation. Microvessel diameter and red blood cell velocity were determined on line by a charge coupled device (CCD) system (Paffhausen & Rosen, 1988).

After systemic application of endothelin (2 - 5 ug/kg i.v.) a shortlasting fall in blood pressure (up to 20 s) occurs prior to an increase which lasts up to 15 min and is dose independent. Arterioles (mean diameter 45 um) constrict by 80 to 100% of the control diameter for more than 30 min. Superfusion of the pouch with acetylcholine (10^{-6} M, ACh) after the injection of endothelin (2 ug/kg) causes a permanent vasorelaxation whereas after 5 ug/kg the arterioles constrict again after the ACh relaxation has dissipated. This procedure can be done repeatedly.

To investigate, whether endothelin constricts microvessels directly we superfused the cheek pouch with endothelin (10^{-11} M to 10^{-8} M). At the lowest concentration the effects are inconsistent after a superfusion time up to 10 min. A 2 min superfusion with 10^{-10} M causes a constriction of 60 to 80%, which lasts 10- 15 min. This can be reversed by ACh (10^{-5} M). Increasing the endothelin superfusion time or its dosage constricts arterioles for more than 30 min and cannot be antagonized by ACh (10^{-5} M). In those vessels dilating due to ACh administration the recovery of the red blood cell velocity was impaired. Interestingly, the repetetive relaxation by ACh appears only for the time of its superfusion.

These data show, that endothelin is a potent constrictor of microvessels in the hamster cheek pouch. The constriction after systemic application remains when the blood pressure increase is normalized indicating a direct microvessel effect beside a reactive vasoconstriction. This is supported by the superfusion experiments, showing that the constriction lasts significantly longer than the superfusion. Ach (10^{-5} M) is unable to antagonize the constriction induced by longer exposure or higher concentrations of endothelin. This shows the limitations of endothelium dependent relaxation of endothelin induced microvessel constriction. The ACh effect is limited to its exposure time. The divergence between permanent relaxation and the recovery of the red blood cell velocity may be explained by a massive disturbance of the blood flow distribution within the microvascular network. From our data we conclude that endothelin causes a longlasting vasoconstriction in microvessels even after a shortlasting local application.

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A DEXAMETHASONE-INDUCED INHIBITOR OF IL-1 RELEASE FROM POLYINOSINIC: POLYCYTIDYLIC ACID AND LIPOPOLYSACCHARIDE-STIMULATED MONOCYTES

J. Davidson, A.S. Milton, & D. Rotondo, Department of Pharmacology, University of Aberdeen, ABERDEEN AB9 1AS Scotland, U.K.

We have recently shown that the synthetic glucocorticoid dexamethasone (DEX) is antipyretic toward the synthetic double-stranded ribonucleic acid polyinosinic:polycytidylic acid (Poly I:C), the purified cell-wall component of gram -ve bacteria, lipopolysaccharide (LPS) and native interleukin 1/endogenous pyrogen, (Abul *et al*, 1987). Many biological actions of DEX are thought to be mediated through the induction of lipocortin which can inhibit phospholipase A_2 and hence eicosanoid biosynthesis and this may be involved in the antipyretic action of DEX. Interleukin-1 (IL-1) is thought to be a mediator of fever in response to LPS and Poly I:C and is released from a variety of cell types primarily mononuclear phagocytes such as macrophages and peripheral blood monocytes (Mø). It is not known whether the antipyretic action of DEX involves a decrease in the release of IL-1. We have presently studied the effects of DEX itself and also supernatant from Mø incubated in the presence of DEX on the release of IL-1-like activity from Mø stimulated with LPS (1 μg/ml) and Poly I:C (100 μg/ml).

Mø were prepared from rabbit blood by percoll density gradient centrifugation followed by incubation in plastic tissue culture petri-dishes for 2 hours at 37 °C, 5 % CO_2, 100 % humidity after which the non-adherent cells were discarded and the adherent cells were scraped from the culture dishes and used as the Mø (Rotondo *et al*, 1988). Mø (1×10^6 cells) were incubated for 20 hours and IL-1 activity was measured from the supernatants. IL-1 activity was measured by incubating Mø supernatants with thymocytes (from C3H/HeJ mice) which were incubated with phytohaemagglutinin, pulsed with [^{3}H] thymidine and the incorporation of radioactivity into acid insoluble material measured. Incubations were carried out in RPMI 1640 containing 5 % foetal calf serum and 25 μM mercaptoethanol. The viability of thymocyte incubations was verified by including standards of human recombinant IL-1α and IL-1β obtained from the National Institute for Biological Standards and Control.

DEX produced a concentration-dependant decrease in the release of IL-1-like activity from Mø incubated in the presence of LPS, Poly I:C and media alone. With all three treatments, a significant decrease occurred with a concentration of 2 nM DEX and a maximal inhibition was observed with 2 μM DEX which reduced the incorporation of [^{3}H] thymidine to less than 5 % ± 2.3 % of the control values in the absence of DEX. Experiments were also carried out where Mø were stimulated with 10 μM DEX for 6 hours and the supernatants were collected, passed through Sep-Pak C_{18} columns to remove DEX and dilutions of the eluate were included in incubations with Mø. A decrease in IL-1-like activity occurred with a 1000-fold dilution of the eluate which reduced the LPS and Poly I:C-stimulated IL-1-like activity to 70.6 ± 9.1 % and 81.8 ± 4.8 % of the respective control values and a maximal inhibition was observed with a 10-fold dilution which reduced the IL-1-like activity to 58.6 ± 8.3 % and 66.4 ± 5.9 % of the respective control values. LPS, Poly I:C, DEX and the DEX-stimulated partially purified supernatant did not directly affect the incorporation of radioactivity into acid insoluble material from the thymocytes indicating that the changes in incorporated radioactivity were in response to the Mø supernatants and not as the result of a direct effect on the thymocytes.

These data show that DEX can inhibit the basal release of IL-1-like activity from Mø and from Mø stimulated with LPS and Poly I:C. The results also indicate that the inhibitory activity of DEX occurs via a mediator released into the incubation medium as supernatants obtained from Mø stimulated with DEX, and with the DEX subsequently removed, also inhibited the release of IL-1-like activity from Mø.

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MODULATION BY RENAL INTERSTITIAL CELLS OF THE SLOW PRESSOR RESPONSE TO ANGIOTENSIN II IN THE CONSCIOUS RAT

P.M. Roberts, A.J. Freeman and M.G. Dodds. Cardiovascular Pharmacology Department, Glaxo Group Research Ltd., Greenford, Middlesex UB6 OHE.

The renomedullary interstitial cells (RIC) secrete vasodepressor lipids which may oppose the actions of angiotensin II (AII) in blood pressure homeostasis (Muirhead et al, 1986). Chronic administration of acutely sub-pressor doses of AII causes a slow sustained rise in blood pressure (Diz et al. 1983). We have studied the effects of the RIC toxin 2-bromoethylamine (BEA) (Taverner et al, 1983) on the slow pressor response to AII in conscious rats to determine whether this response is modified by RIC function.

Four groups of 7 male Sprague-Dawley rats (150-180g) received isotonic saline (SAL) or BEA 200 mg kg^{-1} i.p.. Five weeks later a carotid artery was cannulated under pentobarbitone sodium (50 mg kg^{-1} i.p.) anaesthesia to permit direct blood pressure recording. Osmotic minipumps were implanted i.p. to deliver either SAL 0.5 µl hr^{-1} or AII 480 ng kg^{-1} min^{-1} to the SAL or BEA pretreated groups of rats. Blood pressure was recorded daily for 10 days. At the end of the experiment the kidneys were removed for histological examination.

BEA pretreatment alone (Table 1) had no effect on mean arterial blood pressure (MABP). AII caused a rise in MABP starting after 3 days and reaching a plateau after 7 days of infusion. There was no difference in the pressor response to AII in SAL or BEA pretreated rats.

Table 1 Mean arterial blood pressure (mm Hg) Mean ± s.e mean

Group	n	Day 2	Day 3	Day 7	Day 10
SAL/SAL	7	122 ± 3.1	121 ± 1.4	122 ± 3.8	121 ± 4.5
BEA/SAL	7	121 ± 1.8	123 ± 4.2	126 ± 4.2	118 ± 5.0
SAL/AII	7	139 ± 7.8	139 ± 8.1	165 ± 14.3	161 ± 14.6
BEA/AII	7	137 ± 10.0	128 ± 7.1	170 ± 18.5	178 ± 13.9
*BEA/SAL	4	122 ± 3.0	125 ± 3.8	127 ± 2.8	127 ± 2.4
*BEA/AII	4	147 ± 10.8	139 ± 8.6	194 ± 9.4	205 ± 5.0

BEA caused variable RIC damage. Four rats in each of the groups BEA/SAL and BEA/AII had confluent necrosis of the renal papilla which extended to the inner medullary junction; other rats had less marked RIC damage. In the subgroups of rats with confluent necrosis of the renal papilla, BEA alone had no effect on MABP; however AII infusion caused an apparently more rapid rise in MABP with a greater maximum effect than that seen in SAL-pretreated rats, suggesting the response to AII may depend on the extent of RIC damage (Table 1). Regression analysis established that the rate of rise of MABP from days 3-10 in the subgroup *BEA/AII was significantly greater than that in group SAL/AII ($P<0.01$). These results support the proposal that pressor responses to AII may be modified by RIC function.

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ANGIOTENSIN II INDUCES A REDISTRIBUTION OF PROTEIN KINASE C ACTIVITY IN HUMAN PLATELETS

A.L.Sugden & D.Sugden[1], Blood Pressure Unit, Department of Medicine, Charing Cross & Westminster Medical School, London W6 8RF and [1] Division of Biomedical Sciences, King's College London, London, W8 7AH.

Human platelets have a low density of high affinity angiotensin II (AII) binding sites. However, addition of AII to platelets has no direct effect on platelet aggregation but does facilitate aggregation induced by adrenaline and the stable thromboxane A_2 agonist, U44069 (Ding et al., 1985). This response may be of pathophysiological significance as platelet activation in vivo may result from the synergistic action of several agonists. The mechanism involved in this potentiation is unknown but considerable evidence indicates a direct role for protein kinase C (PKC) in secretion and aggregation in platelets. The aim of the present study was to examine the effect of AII on the cellular distribution of PKC in human platelets. A translocation of PKC from cytosol to membrane is thought to reflect enzyme activation, and AII has been shown to increase membrane PKC activity in adrenal glomerulosa cells (Lang & Vallotton, 1987).

Washed human platelets were resuspended in physiological saline containing 2.5 mM EDTA and 100 µg/ml leupeptin. Aliquots of platelets ($1-3 \times 10^8$ cells) were then permeabilized with saponin (10 µg/ml, 1 min.) and treated (37°C) for 1 or 5 min. with saline, AII (10^{-7} M), thrombin (0.5 U/ml) or 12-O-tetradecanoylphorbol 13-acetate (TPA, 10^{-7} M). Platelets were collected by centrifugation (15 s, 12000 x g), disrupted by sonication into buffer A (20 mM Tris HCl, pH 7.5, 0.5 mM EGTA, 2 mM EDTA, 0.1% β-mercaptoethanol, 50 µg/ml leupeptin, 1 mM PMSF) and cytosol and membrane fractions separated by centifugation (30 min., 4°C, 35,000 x g). Membrane-associated activity was solubilised with NP-40 (1% v/v) then PKC activity was partially purified by DE52 anion exchange chromatography and measured in duplicate as described previously (Parker et al., 1984).

TPA, a direct activator of PKC, induced a substantial increase in membrane-bound activity (mean±s.e.mean of 4 experiments): control, 8.0±1.6 nmol/min/mg protein; TPA, 56.2±6.4 nmol/min/mg protein. Treatment of platelets with thrombin did not significantly increase membrane activity, 12.9±3.4 nmol/min/mg protein and 8.5±3.8 nmol/min/mg protein after 1 and 5 min. Although thrombin is known to trigger phosphatidylinositol hydrolysis and activate PKC in platelets (as judged by the phosphorylation of an endogenous 40 kDa substrate) no translocation of PKC activity to the membrane was apparent. Perhaps the explanation lies in the rapid desensitization of the thrombin receptor (Crouch & Lapetina, 1988). AII produced a statistically significant increase ($p<0.05$ using Student's t-test) in membrane-bound PKC activity, 18.1±3.7 nmol/min/mg protein after 1 min. and 15.4±2.6 nmol/min/mg protein after 5 min. of treatment. The increase in membrane-bound PKC activity following AII may be responsible for the facilitation of platelet aggregation in response to adrenaline and U44069.

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EFFECTS OF TISSUE PLASMINOGEN ACTIVATOR (BW t-PA) ON PLATELET FUNCTION IN VITRO AND EX VIVO

H. Giles[+], D.J. Longridge, M.J. Follenfant and B. Hughes, Dept. of Pharmacology and [+]Biochemical Sciences, Wellcome Research Laboratories, Beckenham, Kent, UK.

Thrombolytic agents have been reported to induce both aggregation (Fitzgerald et al., 1988) and disaggregation (Ohlstein et al., 1987) of platelets from several species including man. The purpose of the present study was to evaluate the effects of BW t-PA on human platelet function in vitro and on dog platelets during evolving myocardial infarction (MI) and thrombolytic therapy in a canine coronary thrombosis model.

Platelet rich plasma (PRP) from human blood was prepared by centrifugation of blood drawn into citrate (3.15%) from healthy male (n = 3) and female (n = 3) volunteers (who, for at least two weeks had not taken any drug known to affect platelet function). Platelet aggregation to adenosine diphosphate (ADP) and collagen was recorded as changes in light transmission on a Payton aggregometer. BW t-PA or vehicle (0.9% saline) was added to the PRP two minutes prior to aggregation and results were expressed as percent inhibition (or percent aggregation) of the control aggregation in the absence of t-PA. Ex vivo platelet aggregation (to ADP) was performed in anaesthetised beagle dogs (n = 7) undergoing 90 minutes thrombotic coronary occlusion and 2 hours reperfusion (Longridge et al., 1988). BW t-PA was administered as an infusion to induce lysis (1.0 MIUkg^{-1} over 1 hour) at the end of the 90 minute occlusion period and as a maintenance infusion (0.12 MIUkg^{-1}hr^{-1}) for the 2 hour reperfusion period. Blood (7 mls) was drawn into citrate (2.5%) prior to occlusion, at the end of 90 minutes occlusion, at the end of the lytic infusion of BW t-PA and at the end of the maintenance infusion. PRP was prepared using a rapid spin method as described by Whittle et al. (1980).

On human platelet in vitro, t-PA (300-20,000 IUml^{-1}) alone did not induce shape changes or aggregation nor did it inhibit aggregation induced by a concentration of ADP (2-10μM) or collagen (0.5-1.0 μgml^{-1}) just sufficient to elicit maximum aggregation. Concentration effect curves to collagen and ADP were constructed in the presence of 10,000 Uml^{-1} t-PA or vehicle. t-PA had little or no effect on the collagen responses but there was a small but consistent (<0.2 log unit) rightward shift of the ADP curve. In dogs undergoing evolving MI and administration of BW t-PA (which induced successful lysis, mean plasma level 478 ± 71 IUml^{-1}) no change in the aggregatory response of platelets to ADP (0.3-300 μM) was observed.

This study demonstrates that BW t-PA did not cause any platelet hyperaggregability either on human platelets in vitro (at concentrations up to 60 times that required for thrombolytic efficacy) or on dog platelets ex vivo during coronary thrombolysis and evolving myocardial infarction.

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OESTROGEN INDUCED MYOFIBROBLASTS - A MODEL FOR INVESTIGATING MYOFIBROBLAST CONTRACTILITY

S.E. Cross, K.M. Elased* and I.L. Naylor. Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford, West Yorkshire,BD7 1DP

Studies to determine the mechanism(s) of myofibroblast contractility are hindered by the lack of a suitable animal model. The most extensively used model is the croton oil induced granulomatous tissue (Majno et al., 1973). However, the extrapolation of results from this tissue to human pathological states is questionable since this tissue appears to have a range of agonist sensitivities clearly absent in other animal and human tissues containing myofibroblasts. Ross and Klebanoff (1967) have reported that the administration of oestrogen to sexually immature rats induced the formation of 'atypical fibroblasts' in the myometrium. These 'abnormal' fibroblasts have ultrastructural features in common with myofibroblasts and it was considered of interest to determine the pharmacological sensitivity of these induced cells to allow comparison with data obtained using myofibroblasts from other sources.

Sexually immature CD rats (Bradford strain, n = 62, 21 days old 50-55g) were divided into 2 groups (A & B) of 32 and 30 animals. Group A were controls and received 1ml/kg maize oil:ethanol mixture, injected subcutaneously in the intrascapular region. Group B received a single administration of 1mg/kg oestradiol-17β in the control solvent. 24, 72 and 120 hr after treatment animals were stunned, exsanguinated and both uterine horns removed, weighed then one placed in Bouin's fixative solution and the other arranged for superfusion. Isometric responses to barium chloride (10-640μg), 5-hydroxytryptamine (5-HT) (1-100μg) and mepyramine (10-640μg) were investigated. Responses were calculated as (a) maximal tension produced during response and (b) the area under the response curve using a digital integrator (Illingworth & Naylor, 1982).

Histological examination found that compared with the controls luminal epithelium was significantly ($P<0.001$) thickened at 24 and 72 hrs but returned to control levels at 120 hr. Significant increases ($P<0.01$) in the mitotic index, thickness of longitudinal and circular muscle layers were also found. The effect of oestradiol-17β to modify uterine weight is shown in table 1.

Table 1. The median uterine wet weight (mg) (median body weight is given in parenthesis)

	24 hr	72 hr	120 hr
Control	35.8 (57.0) n = 10	42.4 (68) n = 10	57.4 (78.5) n = 12
Treatment	67.8*** (57.3) n = 8	67.8** (70.5) n = 12	59.2 (77) n = 10

**P<0.01, ***P<0.0001 (Mann Whitney 'U' test)

Superfused tissues taken from treated animals showed a significant ($P<0.01$) decrease in responsiveness (both contraction height and area of the response) to barium ions at 24 hrs but returned to control levels at 72 and 120 hrs. The responses to 5-HT in terms of both contraction height and area were significantly increased ($P<0.001$) at 24 hr but returned to control levels at 72 and 120 hr. Mepyramine contractions in the treated animals, both the tonic and phasic components of the response, were significantly ($P<0.01$) decreased at the 24 and 72hr time interval studied but were the same at 120hr.

The results indicate that in oestrogen stimulated immature rat uterus the depression of the contractile response to mepyramine, the agent shown elsewhere to be a stimulant of myofibroblast contractility, suggests that the uterine tissue is not suitable for in vitro studies of myofibroblast contractility.

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*Present address: Pharmacology Dept., University of Strathclyde, Glasgow

CP-20961-INDUCED ARTHRITIS AND ITS MODULATION IN THE RAT

A.M. Drah and M.C. Henman, Department of Pharmacology, Trinity College, 18 Shrewsbury Road, Dublin. 4, Ireland.

CP-20961 [N,N-dioctadecyl-N,N-bis(2-hydroxy ethyl) propanediamine] has been shown by Chang et al (1980) to induce arthritis in the rat which is essentially similar to classical adjuvant arthritis. Female Wistar rats (190-240g) were injected (day 0) with 2 mg of CP-20961 in 10 ul of Freunds Incomplete adjuvant (FIA) into one inguinal node in each flank while a further 5mg of CP-20961 in 0.1ml FIA was injected intradermally at the base of the tail. Control animals underwent the same procedure except that they received FIA alone. The animals were observed until day 28 and the intermalleolar and foot thickness were measured using caliper on day 0, 14, 21 and 28. At the end of the experiment, spleen and thymus weights were recorded and tissue samples were retained for histology.

CP-20961 caused an increase in the intermalleolar (6.81±0.04, day 0, vs. 9.91±0.18mm, day 28, $P<0.01$) and foot thickness (5.65±0.03,day 0, vs. 8.14±0.12mm, day 28, P0.01). Compared to control animals there was an increase in the spleen weight (260.26±12.37 vs. 347.12±13.33mg/100g body weight,$P<0.01$) and a decrease in thymus weight (119.82±13.22 vs. 70.14±8.20mg/100g body weight, $P<0.01$). Removal of the inguinal nodes on day 5 after CP-20961 administration prevented the appearance of arthritis as there was no significant increase in the intermalleolar and foot thickness, nor were the spleen or thymus weights significantly different from control animals. In contrast, removal of the inguinal nodes on day 14 after CP-20961 administration did not alter the progression or decrease the severity of the arthritis as measured by increased intermalleolar (6.87±0.03, day 0, vs. 9.85±0.29 mm, day 28, $P<0.01$) and foot (5.57±0.03, day 0 vs. 8.01±0.26mm, day 28, $P<0.01$) thickness. Spleen weight was greater than in control animals ($P<0.01$) and thymus weight was less ($P<0.05$). Cyclosporin A administration (5mg/kg, p.o.) daily from day 0 for 10 days reduced the extent of swelling and the spleen and thymus weight did not differ significantly from control animals. On established arthritis the same dose of cyclosporin A had much less effect on the increase in intermalleolar (6.82±0.04, day 0, vs. 8.86±0.16mm, day 28, $P<0.01$) and foot (5.66±0.04, day 0, vs. 7.26±0.19mm, day 28, $P<0.01$) thickness. The thymus weight was less than controls ($P<0.01$) and there was no significant change in the spleen weight.

These results suggest that CP-20961 must be processed in some way in order to induce arthritis and that the processing of the adjuvant is more sensitive to immunosuppression than in established arthritis.

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A STUDY OF THE ACTIVITY OF 9α11β-PGF_2 AT PROSTAGLANDIN RECEPTORS

H. Giles, M.L. Bolofo, S.J. Lydford & G.R. Martin, Analytical Pharmacology Group, Biochemical Sciences, Wellcome Research Labs., Langley Court, Beckenham, Kent, BR3 3BS.

9α11β - prostaglandin(PG)F_2, a major metabolite of PGD_2, has been reported to have numerous biological effects (Liston and Roberts 1985, Pugliese et al 1985), although its site(s) of action has not been described. In this study we have attempted to identify the prostaglandin receptor(s) involved in the responses to 9α11β-PGF_2.

DP and IP-receptor activity was assessed by measuring inhibition of ADP-induced human platelet aggregation using washed platelets (WPS) and platelet-rich-plasma (PRP). Actions at other prostanoid receptors were evaluated using isolated segments of rabbit jugular vein (RbJV, TP-receptors), cat trachea (EP_2), guinea pig ileum (EP_1) and cat iris sphincter (FP), as described elsewhere (Coleman 1987, Giles et al 1989). Platelet experiments were performed in the presence of BM13.177(10μM), a TP-receptor antagonist, and experiments measuring smooth muscle contraction were carried out in the presence of BW A868C (0.1μM) a recently described selective DP-receptor antagonist (Giles et al 1989).

9α11β-PGF_2 (0.3-10μM) caused inhibition of platelet aggregation. Lack of potency prevented the construction of full concentration-effect curves but 10μM 9α11β-PGF_2 caused 70% and 30% inhibition of aggregation in PRP and WPS respectively. These effects were abolished by BW A868C (0.1μM). In the presence of BW A868C inhibition of aggregation by PGI_2 was unaffected by prior addition of 10μM 9α11β-PGF_2. When DP-receptor effects were blocked (by BW A868C) 1-10μM 9α11β-PGF_2 caused a platelet shape change which was blocked by BM13.177. TP-receptor activation was confirmed in the RbJV where 9α11βPGF_2 (in the presence of 1μM BW A868C) caused dose-dependent contractions,([A_{50}] = 0.6μM) which were antagonised in an apparently competitive manner by BM13.177, and reached a similar maximum response to that attained by the TP-receptor agonist, U46619.

When TP-receptors were blocked with BM13.177 (100μM) 9α11β-PGF_2 (0.3-10μM) caused 15-70% relaxation of histamine-contracted RbJV. These effects were antagonised by BW A868C. On some occasions small BW A868C-resistant relaxations (5-20%) were observed at 10-100nM 9α11β-PGF_2, resulting in a biphasic concentration-effect curve. This more potent action is unlikely to be via the IP-receptor because the platelet data suggest that 9α11β-PGF_2 lacks affinity at this site. However, it might reflect activity at the EP_2-receptor since 9α11β-PGF_2 also caused relaxation of bethanechol-contracted cat trachea.

9α11β-PGF_2 caused contractions in cat iris sphincter ([A_{50}]=50nM) similar in magnitude to those attained by $PGF_{2\alpha}$. Likewise, it contracted guinea-pig ileum ([A_{50}]=1μM), as did the FP-receptor agonist, fluprostenol. In both cases the maximum response was less than 30% of that achieved by PGE_2. In neither tissue were responses antagonised by BM13.177 (10μM) or by the EP_1 receptor antagonist AH6809 (5μM).

The lack of selective antagonists at each receptor type prevents definitive conclusions as to the sites of action of 9α11β-PGF_2. Nevertheless, these results suggest that it behaves as a full agonist at TP and FP-receptors, also has efficacy at DP and EP_2-receptors but has no significant affinity for IP-receptors. Its effects in guinea-pig ileum are not via the EP_1 or TP-receptor, but may be through the FP-receptor since fluprostenol also contracted this tissue. Clearly, this primary metabolite of PGD_2 can activate numerous prostaglandin receptors and may, therefore, contribute to the in vivo biological responses seen following administration of PGD_2.

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ENDOTOXIN-INDUCED PROSTANOID OUTPUT FROM RAT LUNG: LACK OF INHIBITION BY METHYLPREDNISOLONE

T Izumi & YS Bakhle, Department of Pharmacology, Royal College of Surgeons, Lincolns Inn Fields, London WC2A 3PN

Endotoxin (ETX)-induced lung injury in experimental animals has been used as a model of the Adult Respiratory Distress Syndrome (ARDS; Brigham & Meyrick, 1986) and some features of this model are ameliorated by corticosteroids (Borg et al 1985). Recently we found that ETX, given i.p. to rats, produced leucopenia and lung oedema over the subsequent 28h, effects that were largely prevented by methylprednisolone (MP; Izumi & Bakhle, 1988). Here we describe the effects of the same treatments on the output of TxB_2 and 6-oxo-$PGF_{1\alpha}$ from rat isolated lungs.

Male rats (200-250g) were injected once (i.p.) with ETX (*E. coli* 0111-B4 LPS-B, 3.5mg/kg) and the lungs isolated at fixed times up to 28h after ETX, from anaesthetised animals. Lungs were perfused with Krebs solution at 8ml/min via the pulmonary circulation and given a bolus injection of either arachidonic acid (AA; 10µg) or calcium ionophore A23187 (3µg). Lung effluent was collected for the subsequent 5min and its content of TxB_2 and 6-oxo-$PGF_{1\alpha}$ measured by radio-immunoassay. Another group of rats received MP (30mg/kg; s.c.), 30 min after the injection of ETX and a third group MP only, i.e. ETX replaced by saline. For each set of treatments, 6-8 rats were used at each time.

Output of TxB_2 from AA was increased about 15-fold by 2h after ETX, to 3.1±0.28ng/ml from 0.2±0.07ng/ml in untreated lungs ($p<0.05$, unpaired t-test), and recovered slowly to normal by 16h. This pattern was not altered by treatment with MP and, in lung from rats receiving MP only, TxB_2 output was not changed. In contrast, output of 6-oxo-$PGF_{1\alpha}$ fell soon after ETX (4-6h) to a minimum value of 0.16±0.05ng/ml, about 25% of the untreated value, but recovered by 28h. Treatment with MP after ETX prevented this fall and maintained 6-oxo-$PGF_{1\alpha}$ output at or above untreated levels. However, MP only caused a slow but sustained (6-28h) decrease in 6-oxo-$PGF_{1\alpha}$ to about 30% of untreated values.

Using the ionophore to liberate endogenous AA from lung lipids, TxB_2 output was again increased by 3- to 4-fold over 2-6h after ETX. MP treatment decreased this only at 4h, from 0.84±0.12ng/ml to 0.42±0.12ng/ml. In contrast to the results with exogenous AA, output of 6-oxo-$PGF_{1\alpha}$ generated by ionophore was increased after ETX but only at later times (16 and 28h). Treatment with MP decreased 6-oxo-$PGF_{1\alpha}$ output only at 28h. The output of either TxB_2 or 6-oxo-$PGF_{1\alpha}$ from endogenous AA, using the ionophore, was not inhibited by MP only, at any time.

We conclude that although prostanoid output from isolated lung was altered by ETX induced injury *in vivo*, the beneficial effects of MP on this lung injury were not a reflection of decreased prostanoid production and that these prostanoids were not significant causes of the observed lung injury.

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THE EFFECT OF RIOPROSTIL AND BUTAPROST (TR4979) ON HUMAN MYOMETRIUM AND UTERINE ARTERY

J.K. Clayton, K. Marshall, J. Senior, N. Tufeak and P.J. Gardiner[+], School of Pharmacology, University of Bradford, Bradford, BD7 1DP, and [+]Bayer Research, Stoke Court, Stoke Poges, Slough, SL2 4LY

Prostaglandins (PG) produce several different effects on the uterus which depend not only on the actual PG used but also the species under investigation and the experimental conditions. The human uterus has been shown to be highly sensitive to the effects of the PG of the E series. The in vitro myometrial response to PGE_2 is biphasic consisting of an initial dose independent contraction followed by a dose dependent inhibition of spontaneous activity.

Uterine blood flow is very variable, the control mechanisms are complex and are greatly influenced by the phases of the menstrual cycle and pregnancy. On uterine artery PGE_2 evokes only a contractile response (Baxter et al. 1988b). In this study PGE_2 was used as a standard with which to compare the effects of the PGE analogues rioprostil and butaprost (Gardiner 1986) on both myometrial and arterial preparations.

Samples of human ascending uterine artery and myometrium were obtained from pre-menopausal patients at hysterectomy or from pregnant (myometrium only) patients (at term but not in labour) during Caesarean section. The myometrial strips were set up as previously described (Massele and Senior 1981) and were superfused with Krebs solution at 2ml min^{-1}. The artery was carefully cleared of connective tissue and 5mm segments were suspended in an organ bath under 1g tension in Krebs solution at 37°C. Any relaxant effect of the compounds was investigated in the presence of phenylephrine at a concentration that elicited 50% of the maximal response.

Rioprostil produced a response qualitatively similar to that evoked by PGE_2, that is, an inhibitory effect which was related to dose, but was preceded by a non-dose related increase in myometrial tension. However, the PGE_1 analogue butaprost only produced an inhibitory response on myometrial preparations. On uterine artery neither rioprostil nor butaprost evoked any contractile effect.

Earlier studies on human myometrium from this laboratory (Baxter et al. 1988a) supported a role for the EP_2-receptor subtype in the inhibitory response elicited by PGE_2. From the results of this study the relaxant effect of butaprost supports the case for it being a selective EP_2 receptor agonist, this selectivity was also reported by Gardiner (1986). Rioprostil is less selective in that it, like PGE_2, elicits a biphasic response. The dose independent nature of the contractile effect does not allow reliable conclusions to be made, however, from earlier work (Baxter et al. 1988a) it would seem that the EP_1-receptor is not involved in this phase of the response. From the results obtained using human uterine artery it may be concluded that both rioprostil and butaprost do not affect EP contractile receptors.

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DISTRIBUTION OF LIPOCORTINS I, II AND V IN TISSUES OF THE RAT, MOUSE AND GUINEA-PIG

F. Smillie, C. Bolton, S.H. Peers, and R.J. Flower
Pharmacology Group, University of Bath, Claverton Down, Bath, BA2 7AY, UK.

Lipocortins (LC's) are a family of proteins implicated in the regulation of inflammation, the immune response and cell differentiation (see Flower, 1988). Recombinant LC-I inhibits eicosanoid generation (Cirino et al, 1987) and is anti-inflammatory (Cirino et al, 1989) although the full physiological role of this and other LC's awaits clarification. We describe the distribution of three members of the LC family, LC's I, II and V (Pepinsky et al, 1988) in tissues from the rat, mouse and guinea-pig.

Male Wistar rats (300 g) male CFLP mice (35 g) and female Dunkin-Hartley guinea-pigs (750 g) were killed by cervical dislocation and their tissues rapidly removed to ice. The tissues were homogenised in ice-cold PBS containing 0.01 M EDTA and 1% Triton x-100, freeze-thawed to lyse remaining intact cells and re-homogenised. The tissue extracts were boiled with SDS and mercaptoethanol and aliquots containing approximately equal amounts of protein subjected to SDS-PAGE and conventional Western blotting methods using highly specific antibodies raised against recombinant human LC's I and II and against purified rat LC V.

The distribution of the LC's in the various tissues is shown in Table 1, as an arbitary scale of 1-4 based on density of staining on duplicate Western blots. 0 implies no detectable protein. In some tissues especially heart, lung and spleen, LC's I and II appeared as multiple bands, probably reflecting N-terminal degradation of the proteins. In some tissues, the LC I antibody also detected a high molecular weight (~ 68 kDa) protein.

Table 1 distribution of LC's I, II and V

	Rat			Mouse			Guinea-pig		
LC	I	II	V	I	II	V	I	II	V
Heart	3	2	3	2	3	3	2	3	3
Lung	3	3	3	3	3	4	4	4	4
Thymus	3	2	1	2	3	3	0	2	0
Liver	2	1	0	1	2	2	0	1	1
Spleen	4	2	1	3	1	4	3	3	2
Brain	2	2	0	1	2	3	0	1	1
Stomach	2	2	2	2	2	3	2	2	3
Duodenum	0	0	0	0	0	0	0	0	0
Colon	2	1	1	3	3	3	3	3	3
Skeletal muscle	1	2	0	2	1	1	1	2	0

The table shows that the distribution of the different LC's varies both between tissues and species. However, some trends are clear: lung and heart are rich in all lipocortins in all species. Spleen, colon, stomach and thymus also contain substantial amounts of all proteins whereas liver, brain and skeletal muscle contain much less. Curiously, the duodenum appeared not to contain any immuno-reactive proteins at all.

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EFFECT OF STEROIDS ON VASCULAR PERMEABILITY IN RAT LUNGS

D.J. Gould., A.A. Norris., M.E. Leeson., D.M.Jackson. Department of Pharmacology, Fisons plc - Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire, UK.

One proposed mechanism for the therapeutic effects of steroids in asthma is to reduce capillary permeability in the lung (Andersson and Persson, 1988). Considerable evidence has been obtained to demonstrate that acutely administered steroids inhibit vascular leakage in the microcirculation of skin in response to agonists which cause oedema by a direct effect on endothelial cells (Griffiths and Blackham, 1988; Peers and Flower, 1987; Yarwood et al., 1988). We have investigated whether steroids exert a similar effect in the lungs.

Isolated rat lungs were perfused via the pulmonary artery by recirculating (10ml volume) with Kreb's solution (37°C) containing 4% Ficoll 70 at 2 ml/min and were ventilated at 60 breaths/min. Permeability changes were measured by monitoring the leakage of ^{125}I-human serum albumin from the perfusion fluid into the lung tissue and lavage fluid. After a stabilisation period of 10 min, submaximal doses of platelet activating factor (PAF) (1 µg/ml) or leukotriene C_4 (LTC_4) (1 µg/ml) were added to the perfusion fluid reservoir and allowed to circulate for 30 min before the lungs were removed for measurement of oedema.

In another set of experiments, tracheal permeability was assessed in anaesthetised rats given Evan's blue dye (2.0%) i.v. Oedema was induced by the intratracheal injection of 40 µl of 5-HT (100 µg) or by bilateral cervical vagal stimulation (10v/40hz/5msec) for 5 min. Animals were killed 10 min after either stimulus and the tracheal blueing was measured by reading the optical density of the formamide extracted dye at 620nm.

Dexamethasone (3 mg/kg s.c.) was administered to animals 16-20 h before the experiment, in all studies, with a minimum of 5 animals in each group.

Permeability in the isolated lung preparation increased 3.4 ± 1.2 fold with PAF (n = 12) and 4.1 ± 1.1 fold with LTC_4 (n = 6) and neither agonist response was inhibited by dexamethasone. The specific antagonists, WEB 2086 (10 µg/ml) and FPL 55712 (10 µg/ml) added to the perfusate 10 min before the agonists completely inhibited oedema. Tracheal permeability was increased 2.9 ± 0.25 fold with 5-HT (n = 9) and 1.7 ± 0.14 fold with vagal stimulation (n = 8) and dexamethasone was also ineffective at preventing these changes. Five minute pretreatment with methysergide (2 mg/kg i.v.) or one minute pretreatment with morphine (3 mg/kg i.v.) significantly reduced the oedema to 5-HT by 91 ± 5% and to vagal stimulation by 42 ± 16% respectively.

These results indicate that in contrast to the studies carried out with rabbit skin, permeability changes in the lungs and tracheas of rats in response to the above agonists are not inhibited by steroids. These results do not exclude an inhibitory effect of steroids on oedema formation in lungs if circulating neutrophils are involved in the response.

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INVESTIGATION OF PHOSPHOLIPASE-INDUCED PAW OEDEMA IN THE RAT

P. Hambleton & S. McMahon (introduced by P. Miller)
Roussel Laboratories Ltd, Covingham, Swindon, Wilts, SN3 5BZ (UK).

Phospholipase A_2 (PLA_2) and phospholipase C (PLC) are generally regarded as key enzymes in the inflammatory process (Blackwell & Flower, 1983; Vadas & Pruzanski, 1984 ; Bennett, et al 1987). Local administration of PLA_2 has been reported to induce acute inflammation in rabbit skin and rat paw (Pruzanski, et al 1986, Haworth, et al 1988). In the present study we compare PLA_2 with PLC in the rat paw oedema model.

Male CFHB rats (Interfauna, 130-150g) were injected in the hind paw with either cobra venom PLA_2 (Sigma, P.6139,935 units/mg) or C.perfringens PLC (Sigma, P.7633, 12 units/mg). The inflammatory response was measured after 1,3,6 and 24h as paw diameter, after subtraction of the control saline (0.2ml) response in the contralateral paw.

PLA_2 (1-100 units) gave a dose-related response of rapid onset which peaked at 1h, plateaued up to 6h, and resolved by 24h. In contrast, PLC (0.01-0.04 units) gave a dose-related response which increased gradually and peaked at 6h with resolution by 24h. The effect of three pharmacological agents (administered p.o. at -1h) on the 3h responses is shown below.

Compound	Dose (mg/kg)	% change PLA_2 (10u)	% change PLC (0.03u)
Cyproheptadine	10	-55**	+13
Indomethacin	10	- 9	-49**
Phenidone	100	-31*	-30*

* $p < 0.05$, ** $p < 0.005$ (t test)

The histamine H1 and serotonin receptor antagonist cyproheptadine inhibited PLA_2-induced oedema only, the cyclooxygenase inhibitor indomethacin inhibited PLC-induced oedema only, and the joint inhibitor of cyclooxygenase and lipoxygenase phenidone inhibited both responses.

These results suggest, at least for these preparations, that PLA_2-induced oedema was largely due to mast cell-derived products confirming previous reports (Chi, et al 1982; Haworth, et al 1988), whereas PLC-induced oedema was eicosanoid-dependent and mast cell-independent. These data may have implications for the role of these enzymes in more complex inflammatory lesions.

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SYNERGISTIC ACTIONS OF PLATELET-ACTIVATING FACTOR AND 5-HT IN PLATELET AGGREGATION: EFFECT OF 5-HT BLOCKERS AND ANTI-ASTHMA DRUGS

Sheikh A. Saeed, Farah Aleem, *Akhtar Naqvi, *Maqbool H. Jafarey and Amin Suria. Department of Pharmacology, The Aga Khan University, Faculty of Health Sciences, and *Sandoz Pakistan Limited, Karachi, Pakistan.

A number of agents act synergistically to produce platelet aggregation (Saeed et al; 1988). Todate, few studies have been done in human platelets on cooperative effects of platelet-activating factor (PAF) and various agonists. Since most of the circulating 5-HT come from platelets, we have studied the effects of 5-HT and PAF on human platelet aggregation.

Blood was obtained from normal adult volunteers who had not taken any aspirin-like drug for 10 days. Platelet-rich plasma (PRP) was prepared by centrifugation at 260 xg and aggregation was monitored turbidmetrically on a dual-Channel Chrono-Log Aggregometer. All aggregation studies were carried out at 37°C with PRP platelet counts of 2-2.5 x 10^8/ml. The results showed 5-HT upto 500 uM did not induce aggregation ($P < 0.01$, n=50). PAF (50-500 nM concentration) showed a dose-response aggregation in PRP. The subthreshold concentration of PAF was 60±4 nM (n=47). When 5-HT (0.212 mM) and PAF in subthreshold concentrations were added together or successively, a marked potention of aggregation was obtained ($P < 0.01$, n=22). In order to assess the specificity of 5-HT and PAF combination-induced aggregation we tested the effect of various 5-HT receptor blockers on platelet aggregation. Since PAF has been implicated as a mediator of bronchial asthma, we also examined the effect of various anti-asthma drugs against platelet aggregation. The mean values ± S.E.M. (uM) for inhibiting platelet aggregation by 50% (IC50) for various drugs obtained are given in Table 1.

Table 1: INHIBITION OF PAF + 5-HT INDUCED PLATELET AGGREGATION (IC50* (uM) ± S.E.M.)

Drug	Aggregating Agent: Platelet-Activating Factor (PAF)	Aggregating Agent: PAF plus 5-HT	Relative potency against PAF + 5-HT-induced aggregation
Amitriptyline	167 ± 15	0.015 ± 0.0005	11133
Cyproheptadine	2 ± 0.5**	0.035 ± 0.001**	60
Methysergide	52 ± 2	0.06 ± 0.005	870
Methylergometrine	25 ± 1	0.7 ± 0.03	40
Ketotifen	4 ± 0.2	0.7 ± 0.02	6
Sodium Cromoglycate	NI	NI	NI
Aminophylline	NI	250 ± 18	>250
Theophylline	NI	102 ± 28	>>100
Terbutaline	NI	NI	NI
Indomethacin	9 ± 0.7	9 ± 0.5	1
Nordihydroguaiaretic acid (NDGA)	385 ± 5	61 ± 2	6

N.I. Not inhibited * Concentration producing 50% inhibition of aggregation. ** Concentration expressed as nM. Statistical significance was assessed by Students-t-test.

Our data demonstrates that 5-HT potentiates the effect of PAF in inducing platelet aggregation. This effect of 5-HT on aggregation was blocked (Table 1) by 5-HT blockers like amitriptyline, methysergide, cyproheptadine and methylergometrine in a dose-dependent fashion ($P < 0.005$). These drugs also inhibited PAF-induced aggregation but at much higher concentrations. It is noteworthy that indomethacin and NDGA (inhibitors of arachidonic acid metabolism), abolished the potentiating effect of 5-HT on aggregation. Furthermore, the effect of anti-asthma drugs on aggregation show a distinct selectivity for ketotifen in inhibiting PAF/5-HT induced aggregation as compared to cormoglycate which had no effect in this system. These results suggest that increased formation of AA metabolites may be one of the factors responsible for the potentiating effect of 5-HT.

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ACTIONS OF THE PAF ANTAGONIST, BN 52063, ON THE CLINICAL AND HISTOPATHOLOGICAL RESPONSES TO PAF IN ATOPIC SKIN

C.B. Archer[1], A.C. Markey, J. Barker, T.H. Lee[2], & D.M. MacDonald, Departments of Dermatology and [2]Allergy and Respiratory Disorders, U.M.D.S. (Guy's Campus) and [1]Institute of Dermatology, U.M.D.S. (St Thomas' Campus), London.

Platelet activating factor (PAF, Paf-acether, AGEPC) has many properties consistent with a mediator of acute and persistent inflammation. PAF has been identified in cold urticaria (Grandel et al, 1985) and psoriatic scale (Mallet & Cunningham, 1985), and the development of specific PAF antagonists such as the ginkgolide mixture, BN 52063, allows studies of the functional relevance of PAF in cutaneous inflammatory disorders.

We have studied the clinical and histopathological responses to intradermal PAF over a 24 h period in 12 healthy, atopic subjects with no evidence of dermatitis. We also assessed the actions on these responses of oral BN 52063 (120 mg) taken 2 h prior to PAF injection using a randomised, double-blind, placebo-controlled design. Each subject was studied on two occasions, at least one week apart, and on each occasion (BN 52063 or placebo) received two injections of 200 ng PAF, one in each upper arm, and one injection of diluent alone. Weal and flare areas were calculated from two perpendicular diameters, measured at 5, 15 and 30 min after injection. Mean values for the two PAF injection sites on each occasion were calculated. A series of 6 mm punch biopsies was performed at 30 min, 4 h, 12 h and 24 h, each subject being biopsied at the same time on the two study days. Sections were stained with haematoxylin and eosin or chloracetate esterase for counting of inflammatory cells.

Weal and flare responses developed in all subjects following intradermal PAF, with maximal flare area at 5 min and maximal weal area at 15 min. Both components had resolved by 4 h. Unlike in studies of normal skin (Archer et al, 1985), eosinophils were seen as early as 30 min and were maximal 12 h after injection. BN 52063 inhibited flare area responses at 5, 15 and 30 min ($p = 0.02$) but weal area was not signifiantly decreased. There was a significant reduction in the mononuclear cell count at 4 h ($p = 0.01$). A decrease in the total leucocyte count at 4 h did not reach statistical significance ($p = 0.06$).

This confirms that eosinophil recruitment is a feature of atopic skin following intradermal PAF. BN 52063 inhibits some of the clinical and histopathological features of PAF-induced responses in atopic skin.

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EFFECTS OF PLATELET ACTIVATING FACTOR ON CONTRACTILE FORCE AND ^{45}Ca FLUXES IN ISOLATED GUINEA-PIG ATRIA

J. Díez & J. Tamargo. Department of Pharmacology. School of Medicine Universidad Complutense. 28040 Madrid. Spain.

Platelet activating factor (PAF, 1-O-hexadecyl-2-O-acetyl-sn-glycerophosphorylcholine) is a phospholipid which has been implicated in the anaphylactic, immuno complex-mediated and cytotoxic immunopathological reactions (Braquet et al., 1987). In the guinea-pig PAF has been found to reduce coronary blood flow and contractile force (Benveniste et al., 1983) and to increase the amplitude and the Vmax of the slow action potentials (Tamargo et al., 1985), which suggests that it may increase Ca influx via the slow inward current. The present work was undertaken to assess the effects of PAF on contractile properties and ^{45}Ca movements in isolated guinea-pig atria.

Experiments were performed in guinea-pig atria incubated with Tyrode solution maintained at 34ºC and gassed with 95%O_2-5%CO_2. Contractions were isometrically recorded through Grass FT03 transducers on a Grass polygraph. ^{45}Ca uptake and efflux were determined as previously described (Díez & Tamargo, 1987).

In spontaneously beating right atria, PAF (10^{-12}M - 10^{-7}M) produced a dose-dependent positive chronotropic effect ($P < 0.05$) which was accompanied by a shortening in sinus node recovery time ($P < 0.05$). Both in spontaneously beating and electrically driven atria, PAF induced a biphasic effect on contractile force, being positive at low concentrations (10^{-12}M - 10^{-10}M) and negative at concentrations $> 10^{-10}$M. The peak inotropic effect was obtained at 10^{-11}M (+18.8 ± 4.9%. $P < 0.01$), whereas the maximum negative inotropic effect was observed at 10^{-7}M (-15.5 ± 4.4%. $P < 0.01$). At positive inotropic concentrations, PAF potentiated contractile responses to Ca (0.9-9 mM), whereas at negative inotropic concentrations it inhibited them. The reduction in extracellular Na concentration to 70% did not prevent the positive inotropic action of PAF, though it partly reversed the negative one. Verapamil (10^{-6}M) abolished the positive and increased the negative inotropic responses to PAF. At 10^{-7}M PAF also decreased by 26.9 ± 3.2% ($P < 0.05$) the amplitude of the slow contractions induced by isoprenaline in atrial fibres depolarized by 27 mM K. At 10^{-11}M PAF increased ^{45}Ca uptake by 51.8 ± 5.4% ($P < 0.01$) after 10 min of incubation and reduced ($P < 0.05$) the rate coefficient, k_{cm}, for the ^{45}Ca efflux. However, PAF, 10^{-7}M, modified neither ^{45}Ca uptake nor efflux in atrial muscle.

All these results suggest that PAF exerts a biphasic effect in guinea-pig atria. At concentrations $\leq 10^{-10}$M the positive inotropic action might be explained through an increase in Ca influx via the slow inward current or through a decrease in Ca efflux, whereas at higher concentrations an inhibitory effect on the slow Ca channel could be responsible for its negative inotropic action.

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EFFECTS OF SL 81.0433 ON MODELS OF GASTRIC ULCERATION IN THE RAT

M. Prouteau, M. Magnier, M. Delahaye, C.N. Berry & K.G. Lloyd, SYNTHELABO RECHERCHE (L.E.R.S.), 23-25 Ave Morane Saulnier, 92366 Meudon-La-Forêt Cédex, France.

SL 81.0433 (4[[(5-chloro-2-hydroxy-3 propylphenyl)(4-chloro-phenyl)methylene] amino] butanamide) was synthesised as part of an anticonvulsant programme. Secondary pharmacological tests showed SL 81.0433 to prevent stress-induced ulcers. It was therefore decided to explore the antiulcer properties of this compound in various rat models in comparison with known antiulcer compounds (Table).

Gastric lesions were induced in fasted (20-48 hrs) female Wistar rats by: stress (restraint at 4°C for 3 hrs), phenylbutazone (200 $mg.kg^{-1}$ p.o. 2 hrs before sacrifice), ethanol (50%, 1 ml/rat, 1 hr before killing) and pyloric ligature (18 hrs). The animals were treated with either the test compound or vehicle (1% Tween 80) 30 min before or at the same time as (stress, pyloric ligature) the lesion-inducing stimulus. The animals were killed, the stomachs excised and the macroscopic lesions were scored. The antiulcer action of the compounds was expressed as the dose required to reduce the ulceration index by 50% (ED_{50}). The effects of SL 81.0433 compared to cimetidine on gastric acid secretion were studied. Basal gastric acid secretion was measured in conscious rats (Bouclier et al, 1983) and acid secretion induced by histamine, pentagastrine, carbachol and vagal nerve stimulation was studied in anaesthetised rats by perfusing the gastric lumen in situ with sodium hydroxide (2.5×10^{-4} N, 2 $ml.min^{-1}$) and measuring the pH of the pyloric outflow.

As shown in the table, SL 81.0433 inhibited lesion formation in all four models with ED_{50} s ranging from 30-60 $mg.kg^{-1}$ p.o. which compares favourably with sucralfate and carbenoxolone. Cimetidine was considered to be inactive in the ethanol and pyloric ligature models. SL 81.0433 up to 100 $mg.kg^{-1}$ i.p. had no significant effect on basal gastric acid secretion in conscious rats up to 6 hrs after administration except for a significant (30%) decrease at one time point (5 hrs) at 100 $mg.kg^{-1}$ i.p., whereas cimetidine at 30 $mg.kg^{-1}$ i.p. led to significant decreases in gastric acid secretion of 68, 36 and 52%, 1, 2 and 3 hrs respectively after treatment. Cimetidine at 10 $mg.kg^{-1}$ i.p. inhibited gastric acid secretion induced by histamine, pentagastrin and vagal stimulation by between 79 and 90% but was much less active against carbachol-induced secretion (100 $mg.kg^{-1}$ i.p. giving only a 38% decrease). SL 81.0433 had no effect at up to 100 $mg.kg^{-1}$ i.p. on stimulated acid secretion.

Table: Antiulcer activity of SL 81.0433

Compound	Estimated ED_{50}s $mg.kg^{-1}$ p.o.			
	Ethanol	Stress	Phenylbutazone	Pyloric ligature[1]
SL 81.0433	60	30	50	50
Cimetidine	>100	15	3	>100
Sucralfate	400	NT	150	>300
Carbenoxolone	75	>300	200	30

[1]Drugs administered i.p., NT= not tested.

We conclude that SL 81.0433 possesses antiulcer properties independent of an action on gastric acid secretion.

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DETERMINATION OF GLUTATHIONE CONTENT AND MYELOPEROXIDASE ACTIVITY IN RAT MODELS OF INDUCED RECTOCOLONIC INFLAMMATION

K.H.Peh, B.Y.C.Wan, and D.V.Parke*. Pharmacology Department, Biorex Laboratories Ltd., Canonbury Villas, London N1 2HB. *Biochemistry Department, University of Surrey, Guildford, Surrey GU2 5XH.

Reactive oxygen metabolites may mediate mucosal inflammation in active ulcerative colitis (Grisham & Granger, 1988). Glutathione (GSH) is important in the detoxication of oxygen radicals, but its role in the inflamed rectocolon is not clear. We have studied the GSH content (index of oxidant stress) and myeloperoxidase activity (MA, index of inflammation) in rat rectocolonic inflammation induced by ethanol, ethanol+DEM (diethylmaleate - depletor of tissue GSH content), or ethanol+TNBS (2,4,6-trinitrobenzenesulphonic acid, a hapten).

Male Biorex Wistar Rats (190±10g) were fasted, anaesthetised (ether), and given enemas:- groups A and D vehicle, groups B and C 0.25ml 30% ethanol, group E 0.25ml 30% ethanol + 20mg TNBS concomitantly. Group C was given concomitant injection of DEM (1g/kg, s.c.). At 4h (groups A, B,C) and day 14 (groups D, E), the rats were killed by cervical dislocation and two specimens cut along the whole excised length of each distal rectocolon (10cm) were obtained for assay of GSH content (Ellman, 1959) and MA (Krawisz et al, 1984). The results (means ± sd, n=6) are shown below.

Group	A. Control	B. Ethanol	C. Ethanol + DEM	D. Control	E. Ethanol + TNBS
GSH Content (μmol/g tissue)	1.0 ± 0.2	0.8 ± 0.3	0.3 ± 0.1 [1]	1.2 ± 0.4	0.9 ± 0.4
MA ($x10^{-2}$ absorbance unit per min/g tissue)	11.8 ± 10.0	21.4 ± 12.5	8.6 ± 1.8 [2]	6.5 ± 2.3	13.6 ± 8.3 [3]

Mann-Whitney U Test: [1] $p<0.001$ from A & B; [2] $p<0.05$ from B; [3] $p<0.05$ from D.

A separate experiment with similar conditions to groups A to C was performed for macroscopic lesion examination, carried out blind by an independent observer, and scored according to the severity of damage and inflammation (0=normal, 1=mild, 2=moderate, 3=severe, 4=very severe). The scores (A) 0.3±0.5, (B) 2.7±0.5***, and (C) 3.0±0.6*** (n=6, mean ± sd, *** $p<0.001$ from A -- Mann-Whitney U Test).

Ethanol (B) or ethanol+TNBS (E), caused a decrease in GSH content and an increase in MA accompanied by erosions. Ethanol+DEM (C) caused a marked decrease in GSH perhaps resulting in a reduction of LTC_4 formation and thus less inflammation and MA. However, this cannot explain the presence of marked erosion formation and the results in group B. Alternative explanations are that there is a balance between the involement of GSH in oxygen radical detoxification and LTC_4 formation, or a non-specific action of DEM on protein synthesis (Costa & Murphy, 1986). The role of GSH, MA, and LTC_4 in relation to rectocolonic damage remains to be evaluated further.

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INHIBITION OF PAF-INDUCED GASTRIC EROSIONS BY SALMON CALCITONIN

K.L. Andrews, H. Mason, R.F.L. Bates, G.A. Buckley. Department of Life Sciences, Trent Polytechnic, Nottingham.

Platelet-activating factor (PAF) is an endogenous phospholipid implicated as a mediator of ulceration in the stomach. Indeed, intravenous infusion of PAF results in the formation of haemorrhagic erosions in the gastric mucosa of the rat (Rosam et al, 1986). There is now evidence that endotoxin-induced erosions are PAF-mediated (Wallace et al, 1987), and that this mechanism also causes hypotension (Whittle et al, 1986). Salmon calcitonin (sCT) has been found to inhibit gastric erosions induced by a number of ulcer models including, indomethacin (Bates et al, 1979a), restraint stress (Bates & Barlet, 1974) and acetic acid (Bates et al, 1979b). sCT has been found however, to have no beneficial effect on endotoxin-induced hypotension (Bates et al, 1986). In this experiment, the effect of sCT on PAF-induced erosions and hypotension has been investigated.

Male Sprague-Dawley rats (200-250g), were starved overnight before being anaesthetised with urethane (1.6g kg^{-1}). The jugular vein and carotid artery were cannulated for drug administration and measurement of arterial pressure respectively. After a 20 minute stabilisation period, PAF (100ng kg^{-1} min^{-1}) or saline vehicle (control#) was infused for 20 minutes. One hour later, the rats were killed and the stomachs removed and opened in order to count the number of erosions produced. Various doses of sCT (U kg^{-1}) or saline were intravenously administered immediately prior to the beginning of the PAF infusion. Systemic arterial pressure was monitored continuously throughout.

Table 1 The effect of various doses of sCT (U kg^{-1}) or saline on PAF-induced gastric erosions in the rat

sCT (U kg^{-1})	No. ulcers	% inhibition	MAP (mm Hg) time 20 mins
control#	0.67 ± 0.33*	/	-3.5 ± 5.5*
0	6.57 ± 0.37	/	-39.3 ± 1.8
1	5.86 ± 0.46	15%	-41.7 ± 3.0
10	2.57 ± 0.81*	69%	-35.6 ± 3.1
50	1.83 ± 0.79*	81%	-32.5 ± 4.0

* $p<0.005$ (Mann-Whitney) compared to OU kg sCT. Values expressed as mean ± SEM

The results were analysed by the Mann-Whitney U test. sCT (1 - 50 U kg^{-1}) produced a dose-dependent inhibition of PAF-induced gastric erosions, but did not have any effect on PAF-induced hypotension.

We therefore conclude that sCT exerts a powerful inhibition of gastric erosions induced by PAF, but the effect is independent of changes in the systemic arterial pressure.

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PAF-INDUCED BRONCHIAL HYPERRESPONSIVENESS IN THE RABBIT: CONTRIBUTION OF PLATELETS AND AIRWAY SMOOTH MUSCLE

A.J. Coyle, D. Spina, & C.P.Page, Dept of Pharmacology, King's College, University of London, Manresa Road, SW3 6LX.

Platelet activating factor (PAF) is of particular interest in the context of bronchial asthma as it will induce bronchial hyperresponsiveness (BHR) in both experimental animals and man (Page, 1988). As many of the biological properties of PAF are platelet dependent, we have investigated whether the ability of PAF to induce both acute bronchoconstriction and enhanced airway responsiveness are platelet dependent processes. NZW rabbits were anaesthetised with a mixture of Valium (5 mg/kg; i.p.) and Hypnorm (0.4ml/kg; i.m.) and lung function calculated using a Buxco automated respiratory analyser. Airway responsiveness was assessed by exposure to increasing concentrations of histamine (5.0-160 mg/ml) generated by an ultrasonic nebuliser prior to, and 6, 24 and 72 hours after exposure to an aerosol of PAF (80 ug/ml) or 0.25% BSA. Airway responsiveness was calculated as the dose of histamine required to increase lung resistance (RL) by 100% (PC100). 6 and 24 hours after PAF exposure there was a significant increase in BHR (Pre PC100 55 mg/ml; GM n=6, 6hr PC100 14.9 mg/ml; GM n=4, $p< 0.05$; 24hr PC100 21.8 mg/ml; GM n=5, $p<0.05$). 72 hours after PAF exposure there was no significant change in responsiveness compared to Pre challenge (PC100 45.4 mg/ml; GM n=4). Exposure to an aerosol of BSA failed to induce any change in airway responsivness. In another series of experiments, animals were treated with 0.5ml/kg i.p. of a selective antiplatelet serum (APS) administered 16 hours prior to PAF challenge inducing 94 $\pm$ 3% thrombocytopaenia. Normal guinea-pig serum was administered in an identical manner and served as a control treatment. Airway responsiveness to histamine was determined 24 hours later. As above, PAF induced BHR 24 hours after exposure (Pre PC100 58.2 mg/ml GM; Post PC100 19.3 mg/ml GM $p=0.027$ n=6). In contrast there was no significant change in responsiveness in animals renderered thrombocytopaenic (Pre PC100 56.0 mg/ml; Post PC100 54.0 mg/ml GM $p=0.86$). Exposure to an aerosol of 0.25% BSA in control animals or those treated with APS failed to induce any change in BHR. In addition, intrapulmonary bronchi were removed from the lungs of rabbits 24 hours after exposure to either PAF of BSA and suspended in an organ bath containing Krebs-Henseleit solution aerated with 95% 02 and 5% CO2 at 37 C under 1g tension. Cumulative dose response curves to either methacholine or histamine were performed. There was no significant difference in the potency (pD2= log10EC50) to methacholine from bronchi removed from BSA or PAF treated animals (-5.718 ± 0.071 vs -5.774 ± 0.149 respectively n=5, $p>0.05$). The maximum response (mg) was also not significantly different in the two groups of animals (1037 ± 210 vs 1023 ± 179 respectively, n=5, $p>0.05$). Similarly there was no significant difference in either the contractile potency and the maximum response to histamine in bronchi removed from BSA or PAF treated animals (-5.094 ± 0.154 vs -4.974 ± 0.159, n=5, $p> 0.05$; 765 ± 167 vs 864 ± 241, n=5, $p>0.05$ respectively). Thus aerosol administration of PAF induces a platelet dependent bronchoconstriction and BHR. Such changes occurred irrespective of changes in smooth muscle function and may be related to the ability of PAF to induce airway inflammation.

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PAF-ACETHER UPTAKE AND LYSO PAF FORMATION IN THE ISOLATED PERFUSED LUNGS OF OXYGEN-TREATED RATS

E.N. Court & W.P. Kingston, School of Pharmacology, Life Sciences Building, Sunderland Polytechnic, Tyne and Wear SR1 3SD.

We have previously detected PAF-acether in the bronchoalveolar lavage (BAL) fluid from rats which had been exposed to 100% oxygen (O_2) for 12h. Exposure to O_2 for 24h and 48h elicited only changes in lyso PAF levels (Court & Kingston, 1987). The reason why PAF-acether was only detectable at 12 h could be due to an interplay of release, uptake and metabolism. Therefore we have studied PAF-acether uptake and lyso PAF formation in isolated perfused lungs from O_2 treated rats.

Male Wistar rats (250-300g) were exposed to O_2 as described by Court & Kingston (1987) for periods of either 12h, 24h or 48h. A control group of rats (Oh) was not exposed to 100% O_2. The lungs were excised and perfused through the vasculature as previously described (Court & Kingston, 1988). The perfusing fluid was Greenborg-Bohr Buffer containing (0.25% w/v) bovine serum albumin (GBB-BSA). 1.71 nmol PAF-acether or the control (GBB-BSA) was infused at 0.19 ml/min over a total period of 4 min, the perfusate was collected for 19 min from the commencement of PAF-acether infusion. PAF-acether and lyso PAF were extracted from the perfusate and assayed as described by Court etal (1987).

Table 1

PAF-acether (pmol) and Lyso PAF (pmol) content of the perfusate of isolated perfused rat lungs after infusion of 1.71 nmol PAF-acether or GBB-BSA into the vasculature. The results are expressed as the mean ± s.e. mean (n=4).

	Substance detected	Period of oxygen exposure (h) 0	12	24	48
GBB-BSA infusion	PAF-acether	0	21.6±4.4	11.5±0.19	0
	Lyso PAF	41.4±8.3	1281±90	1002±153	910±70
PAF-acether infusion	PAF-acether	510±92	531±83	470±96	560±88
	Lyso PAF	1.23±0.77	370±51	235±86	27.3±12

The amount of PAF-acether detected within the perfusate from PAF-acether infused lungs was not significantly different ($p>0.05$) between the Oh, 12h, 24h and 48h O_2 exposed animals (Table 1). The levels of lyso PAF were significantly decreased ($p<0.05$) in lungs into which PAF-acether was infused compared with lungs infused with GBB-BSA. This finding was similar whichever period of O_2 exposure was examined. PAF-acether infusion may thus have a feedback inhibition on lyso PAF release.

This study indicates that there was no significant difference between the uptake of exogenous PAF-acether or its breakdown to lyso PAF between rats exposed to 100% O_2 for 12h, 24h or 48h.

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EFFECTS OF EPITHELIAL REMOVAL AND CHOLINESTERASE INHIBITION ON RESPONSES OF GUINEA-PIG TRACHEALIS TO MUSCARINIC AGONISTS

J.S. Dixon[1], D.M. Good[2]*, J.A. Gosling[1], & R.C. Small[2], Departments of [1]Cell and Structural Biology and [2]Physiological Sciences, University of Manchester, M13 9PT.

In guinea-pig trachealis treated with indomethacin (2.8μM), removal of the epithelium caused significant leftwards shifts of the log. concentration/effect curves of acetylcholine (ACh; 3.3 fold) and methacholine (MeCh; 2.09 fold) but did not modify curves for carbachol (CCh) or bethanechol (BeCh). Since CCh and BeCh are esters of carbamic acid and hence are resistant to esterase attack, we sought to test the hypothesis that epithelial removal might potentiate ACh and MeCh simply by removing a source of cholinesterase activity.

Pretreatment of indomethacin-treated trachea with ecothiopate (100nM) for 15 min (Flavahan et al., 1985) potentiated ACh and MeCh but did not modify the actions of CCh and BeCh. Removal of epithelium from ecothiopate-treated tissues again resulted in leftwards shifts of log concentration/effect curves of ACh and MeCh but did not modify the curves of CCh and BeCh.

Using isolated guinea-pig ileum as a bioassay system, samples of epithelial scrapings and of guinea-pig whole blood were tested for their ability to hydrolyse ACh. Blood, but not epithelial scrapings, accelerated the hydrolysis of ACh.

Histochemical staining for cholinesterase enzymes (as summarised by Pearse, 1972) confirmed that the epithelial layer was virtually devoid of enzyme activity. Acetylcholinesterase-positive nerve fibres were observed within the submucosa, amongst the trachealis cells and in the adventitia. Pseudocholinesterase activity was prominent in the smooth muscle cells forming the trachealis. Collectively these findings suggest that removal of cholinesterase activity is unlikely to account for the selective potentiation of the acetic choline esters which occurs on epithelial removal.

While epithelial removal did not significantly modify log concentration/effect-curves of CCh and BeCh, it significantly abbreviated the time course of tension development in response to the EC50 of all four choline esters (ACh, MeCh, CCh and BeCh). The latter effect might represent the removal of a diffusion barrier for the cholinesters but selective potentiation of the acetic esters could reflect additional, differential effects of the cholinesters on the release (Goldie et al., 1988) or action of an epithelium-derived relaxant factor.

D.M.G. is an SERC CASE scholar.

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MODULATION OF ^{45}Ca-UPTAKE INTO GUINEA-PIG TRACHEAL CARTILAGE: A COMPARISON OF DIRECT AND INDIRECT METHODS OF MEASUREMENT

P. Gupta, A. Markham, R.M. Morgan and A.J. Sweetman.* School of Pharmacology, Sunderland Polytechnic, Sunderland, SR1 3SD, U.K. *John Dalton Faculty of Technology, Manchester Polytechnic, Manchester, M1 5GD, U.K.

Removal of cartilage from guinea-pig trachealis preparations has been shown to reduce ^{45}Ca-uptake (Raeburn & Rodger, 1984) and alter tissue responsiveness to K^+ and histamine (Raeburn et al, 1987). We now report the ability of certain modifiers of calcium (Ca^{2+}) transport to alter ^{45}Ca-uptake into cartilage rings (CR) using both indirect and direct methods.

Trachae from Dunkin-Hartley guinea-pigs of either sex (700-1000g) were rapidly removed and the C-shaped cartilage rings (CR) dissected free of adjoining muscle. Groups of three rings were weighed and incubated in Krebs-Henseleit Solution (KHS), gassed with 5% CO_2 in oxygen, at 37°C for 15 min. When present, test compounds were added to the incubation KHS, prior to tissue. After 15 min incubation, CR were removed from the medium, blotted, and transferred into KHS in the presence or absence of test compound ^{45}Ca (0.5μCi ml^{-1}), gassed with 5% CO_2 in oxygen at 37°C, giving a final volume of 2 ml. For experiments involving La^{3+}, a modified Tris-KHS ($HCO_3^-/H_2PO_4^-$-free) buffered at pH7.4 and gassed with 100% O_2 was used. Direct ^{45}Ca-uptake was determined following removal of CR from medium, blotting and tissue solubilization in 0.5 ml Soluene-350^R at 60°C for 60 min. Indirect uptake was assessed by removal of 150μl aliquots from the bathing medium. ^{45}Ca content of the solubilized fraction or aliquot was measured using a Beckman LS 7500 Liquid Scintillation Counter. Results are the mean of at least 4 different experiments ± s.e. mean.

Studies using both direct and indirect methods confirmed ^{45}Ca-uptake, statistical analysis confirming significant ($p<0.05$) differences between the methods. ^{45}Ca uptake measured directly increased to 1478 ± 103 fmol ^{45}Ca mg $cartilage^{-1}$ and indirectly to 6233 ± 1033 fmol ^{45}Ca mg $cartilage^{-1}$ after 120 min incubation. All subsequent data refer to a 30 min incubation period. Reducing the incubation temperature to 0-4°C significantly decreased the amount of ^{45}Ca uptake from 1098 ± 46 to 520 ± 35 fmol ^{45}Ca mg $cartilage^{-1}$ when measured directly ($p<0.0005$), and from 2587 ± 177 to 730 ± 118 fmol ^{45}Ca mg cartilage when measured indirectly ($p<0.005$). However boiling of CR or the inclusion of iodoacetate (100μM), diflunisal (100μM), sodium orthovanadate (500μM), diltiazem (10μM), verapamil (10μM) or BAY K8644 (10μM) in the medium failed to alter ^{45}Ca-uptake into CR measured by both methods ($p>0.05$).

When KHS was replaced by Tris-KHS medium, the amount of ^{45}Ca-uptake when measured indirectly was significantly ($p<0.0005$) reduced to 702 ± 162 fmol ^{45}Ca mg $cartilage^{-1}$. The inclusion of La^{3+} (1-10mM) was found to produce concentration-dependent effects, 10mM La^{3+} significantly reducing uptake from 1010 ± 36 to 269 ± 25 fmol ^{45}Ca mg $cartilage^{-1}$ when measured directly ($p<0.0005$), while indirect studies revealed an apparent increase in uptake to 1455 ± 222 fmol ^{45}Ca mg $cartilage^{-1}$ ($p<0.05$).

The data presented indicates that ^{45}Ca uptake is dependent on the following : : a) method employed b) medium and c) is related to passive electrostatic attraction and not active or ion channel mechanisms.

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BRONCHOCONSTRICTOR ACTION OF PGD_2 IN GUINEA-PIGS: EFFECT OF THE DP-RECEPTOR ANTAGONIST BW A868C

S. Hamid-Bloomfield, A.N. Payne, A.A. Petrovic & B.J.R. Whittle. Department of Pharmacology, Wellcome Research Laboratories, Beckenham, Kent, BR3 3BS.

PGD_2 is the major prostanoid released from human pulmonary mast cells following immunological activation in vitro (Holgate et al., 1984). In asthmatics, PGD_2 is released in large amounts into the airway lumen following local antigen challenge (Murray et al., 1986) and is a potent bronchoconstrictor by inhalation (Hardy et al., 1984). In the present study, we have investigated the bronchoconstrictor action of inhaled PGD_2 in anaesthetized guinea-pigs, and the effect of BW A868C (3-benzyl-5-(6-carboxyhexyl)-1-(2-cyclohexyl-2-hydroxyethylamino)-hydantoin), a selective prostanoid DP-receptor antagonist (Giles et al., 1989). In addition, since PGD_2 is known to have contractile effects on airway tissue that are mediated through other prostanoid receptors including those for thromboxane, (Giles & Leff, 1988; McKenniff et al., 1988), we have also investigated the effect of the TP-receptor antagonist BM 13.177 (Patscheke & Stegmeier, 1984).

Male Dunkin Hartley guinea-pigs (400-500g) were anaesthetized with sodium pentobarbitone (60 mg/kg i.p.), mechanically ventilated (54 strokes/min, 1 ml air/100g body wt.) through a mid cervical tracheostomy and pulmonary inflation pressure (PIP) was measured as an index of intrathoracic airway calibre. Aerosols of PGD_2 were generated and administered by direct inhalation using an ultrasonic nebuliser (Lees & Payne, 1986). Systemic arterial blood pressure and heart rate was determined from a cannula in a carotid artery.

In control animals, single 30s administrations of inhaled PGD_2 (0.1 and 1mg/ml), but not vehicle ($NaHCO_3$, 1.25% in saline), provoked a concentration-related rise in PIP with minor cardiovascular effects. Invariably with the higher and in some instances with the lower PGD_2 concentration, the rise in PIP was biphasic. This was evidenced by a transient initial rise of up to 19.3 ± 1 cm H_2O, followed by a smaller but more sustained secondary rise giving a total increase in PIP of up to 26.4 ± 1.4 cm H_2O (mean ± s.e. mean, n = 5). These substantial increases were in marked contrast to a maximum rise in PIP of only 5.5 ± 3.1 cm H_2O (n = 4) following intravenous PGD_2 (2.5 - 160 µg/kg). Pre-treatment with BW A868C (0.1 - 1 mg/kg i.v.) did not significantly reduce the bronchoconstrictor effect of inhaled PGD_2 (n = 5 for each dose). In contrast, pre-treatment with BM 13.177 (2.5 mg/kg i.v.) near-maximally inhibited ($P<0.001$) the first phase of inhaled PGD_2-induced bronchoconstriction and markedly inhibited the secondary phase, the peak rise in PIP provoked by PGD_2 (0.1 and 1 mg/ml), being reduced by 67 ± 5% and 45 ± 6% respectively ($n = 5$, $P<0.01$). Combination of BW A868C (0.1-1 mg/kg i.v.) and BM 13.177 produced no greater inhibition of the bronchoconstrictor effect of inhaled PGD_2 than did BM 13.177 (2.5 mg/kg i.v.) alone (n = 5).

The present results suggest that in guinea-pigs in vivo, DP-receptor activation does not contribute to the bronchoconstrictor effect of inhaled PGD_2, contrasting to the clearly predominant role of TP-receptors. The role of DP-receptors in the bronchoconstrictor effect of PGD_2 in man thus remains to be investigated.

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ATYPICAL BEHAVIOUR OF THE THROMBOXANE ANTAGONISTS EP092, AH23848 AND DALTROBAN IN THE FERRET TRACHEAL RING

M.G. McKenniff, P. Norman, I.W. Rodger[1], & P.J. Gardiner, Bayer Research Stoke Court, Stoke Poges, Slough, Bucks SL2 4LY and [1]Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

It has been suggested that the thromboxane A_2 (TXA_2) receptors that mediate prostanoid-induced contraction of human bronchioles differ from those in human and guinea-pig lung parenchyma and guinea-pig trachealis muscle. The former showing reduced sensitivity to the TXA_2 antagonists EP092 and AH23848 (McKenniff et al, 1988). To determine whether TXA_2 receptors similar to those of the human bronchiolar ring (HBR) exist in the airways of other species, the tracheae from guinea-pig, rat, ferret, hamster, sheep, bovine and rabbit were first tested for sensitivity to the TXA_2 mimetic U46619. The potencies of the TXA_2 antagonists EP092, AH23848 and daltroban (BM13505 Swayne *et al*, 1988) were then assessed. Each airway preparation was suspended in Tyrode's solution containing indomethacin (2.8×10^{-6}M), the experimental protocol was similar to that described previously (McKenniff et al, 1988).

There was wide variation between species in the sensitivity of the airway preparations to U46619 to the extent that some tissues eg sheep and bovine trachea were completely insensitive to U46619. The tissue found to show most similarity to the HBR was the ferret tracheal ring (FTR). The maximum contractile response of the FTR (n = 8) to U46619 was 68 ± 5% (mean ± s.e. mean) of the response to 50mM KCl (0.9 ± 0.23g) compared to 85 ± 4% in HBR. The rank order of prostanoid contractile potency in the FTR was: U46619 >> 16,16diMePGE$_2$ > PGD_2 = fluprostenol = 0. The EC_{50} value for U46619 in the FTR was 8.0×10^{-7}M compared to 2.0×10^{-8}M in the HBR (n = 8). EP092, AH23848 and daltroban (10^{-8} - 10^{-6}M) induced dextral shifts in the U46619 log concentration-response curve. The observed shifts were not however consistent with competitive antagonism, in that the antagonist potency tended to be reduced as the antagonist concentration was increased. Table 1 shows the mean pK_B values calculated at each concentration of antagonist tested.

Table 1 Antagonism of U46619 - induced contractions of the ferret trachea (n=5) by a range of thromboxane antagonists.

ANTAGONISTS	pK_B (± se mean) 10^{-8}M	10^{-7}M	10^{-6}M
EP092	8.0 ± 0.3	7.1 ± 0.2	7.4 ± 0.3
AH23848	8.9 ± 0.2	8.1 ± 0.1	7.4 ± 0.2
Daltroban	8.5 ± 0.3	7.6 ± 0.2	7.0 ± 0.2

The high agonist potency of U46619 in the FTR relative to the other prostanoids would suggest that a TXA_2 receptor is the predominant contractile prostanoid receptor in this tissue. This would appear to be confirmed by the effective antagonism of U46619-induced contractions by the TXA_2 antagonists when tested at 10^{-8}M. However the reduced effectiveness of the antagonists at higher concentrations indicates that the FTR may have a subtype of the TXA_2 receptor, as has been described for the human bronchiole (McKenniff *et al*, 1988).

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EVIDENCE FOR THE PRESENCE OF TWO TYPES OF LEUKOTRIENE D_4 RECEPTORS IN GUINEA-PIG LUNG

N.J. Cuthbert, P.J. Gardiner, P. Norman and T.S. Abram, Bayer Research, Stoke Court, Stoke Poges, Slough, SL2 4LY

Leukotriene (LT) D_4 is a potent contractile agonist of guinea-pig lung parenchymal strips in the presence or absence of indomethacin (Weichman et al, 1982). ICI 198615 has been described as a potent, LTD_4 selective, antagonist on various airway smooth muscle preparations including guinea-pig lung strip. (Snyder et al, 1987). [^{3}H] LTD_4 binding to guinea-pig lung membranes has been shown to correlate to a variety of biochemical and pharmacological effects including thromboxane biosynthesis (Crooke et al, 1987).

We evaluated the effect of ICI 198615 and two less potent leukotriene antagonists, LY 171883 and FPL 55712, on the direct spasmogenic action of LTD_4 (i.e. in the presence of 3×10^{-6}M indomethacin) on guinea-pig lung strips . We also investigated their ability to inhibit the binding of 0.8nM [^{3}H] LTD_4 to guinea-pig lung membranes as described previously (Norman et al, 1984). All three antagonists inhibited [^{3}H] LTD_4 binding in a dose-related manner with affinities (pKi:ICI = 8.85, LY = 6.17, FPL = 5.89) that correlate with their reported pharmacological activities. When tested for their effect on LTD_4-induced contractions of guinea-pig lung strip at 10^{-5}M neither FPL 55712 nor LY 171883 produced any significant change in the concentration-response curve to LTD_4. ICI 198615 (10^{-5}M) produced a dextral shift in the concentration-response curve indicating that it does have some LTD_4 antagonist activity on this tissue; its pK_B is 6.3.

These results suggest that most of the direct spasmogenic effect of leukotrienes on guinea-pig lung strip are mediated through a receptor type on which the present, well-documented, leukotriene antagonists have little or no effect. It is probable that LTD_4 has a lower affinity for this receptor since higher LTD_4 concentrations are required to produce a contractile response in the presence of indomethacin than in its absence. This could explain the failure to observe a site corresponding to this receptor in binding studies with [^{3}H] LTD_4.

Guinea-pig lung strip has at least, two types of leukotriene receptors which differ in agonist, and antagonist, sensitivity. Existing selective LTD_4 antagonists are effective only at the high affinity site and have little or no activity on the direct smooth muscle activity of LTD_4. In contrast this response,in the absence of cyclo-oxygenase inhibitors, is competitively antagonised by ICI198615 with a pA_2 of 9.5 (Snyder et al, 1987).

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BRONCHODILATOR EFFECTS OF CROMAKALIM AND ENPROFYLLINE IN ANAESTHETISED CATS

Ian J. Drewett and Ian W. Rodger, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

Alkyl xanthine drugs are widely used as bronchodilators. The K^+ channel activator cromakalim (BRL 34915) is a recently developed smooth muscle relaxant (Weir and Weston 1986) which may have potential in the treatment of asthma (Allen et al 1986). The in vivo mechanism(s) of action of each of these agents is uncertain. In this study we have compared the effects of cromakalim and enprofylline on airways resistance (R_{aw}) and dynamic lung compliance (C_{dyn}) in anaesthetised cats, and examined whether these effects are synergistic with those induced by isoprenaline.

Methods were essentially those described by Rodger (1974). R_{aw} and C_{dyn} were computed using a Buxco pulmonary mechanics analyser (Model 6). Mean resting values (±s.e. mean) of R_{aw} and C_{dyn} were 5.3±0.5 $cmH_2Oml^{-1}s^{-1}$ and 11.5±1.0 $mlcmH_2O^{-1}$ respectively (n=14). Bronchoconstriction was induced by adjusting the i.v. infusion of 5-HT to produce an increase in R_{aw} of approximately 550% (547±24, n=40), and a decrease in C_{dyn} of approximately 35% (35±2, n=40). Test drugs were administered i.v. to produce cumulative dose-response curves. Results were calculated as percentage reversal of 5-HT effect, and are presented below.

Table 1. Doses (mean ± s.e) of isoprenaline, cromakalim and enprofylline producing a 50% reversal of the 5-HT induced effects.

Drug	Dose producing 50% reversal of 5-HT effect (μg/Kg)	
	R_{aw}	C_{dyn}
(-)-isoprenaline	0.0094±0.0018 (n=9)	0.25±0.14 (n=8)
cromakalim	32±7 (n=5)	70±23 (n=4)
enprofylline	6100±2000 (n=3)	7800±1900 (n=3)

The three drugs produced dose-dependent reversals of 5-HT-induced effects on R_{aw} and C_{dyn}. The rank order of bronchodilator potency was (-)-isoprenaline >> cromakalim >> enprofylline. Time taken to achieve peak bronchodilator response after injection of either (-)-isoprenaline or enprofylline was 30-60s. In contrast, cromakalim required 30-180s. Effects of cromakalim on C_{dyn} were highly variable. A dose of propranolol which produced an 86-fold shift in the dose response curve for (-)-isoprenaline on R_{aw}(50% reversal increased from 7.25 to 625 $ngKg^{-1}$) did not significantly alter the position of either the enprofylline or cromakalim dose-response curves. Bronchodilating doses of either cromakalim or enprofylline failed to potentiate the effects of (-)-isoprenaline.

Since neither cromakalim nor enprofylline potentiates the effects of the ß-agonist (-)-isoprenaline, it is suggested that the in vivo mechanism of action of these drugs, at the doses used, does not involve increased intracellular accumulation of cyclic AMP.

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RELAXATION BY PROPYLENE GLYCOL OF ISOLATED TRACHEAL CHAIN PREPARATIONS

R.D. Rakhit and Helen L. Leathard, Department of Pharmacology, Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF.

Propylene Glycol (PrG) is a supposedly inert, low molecular weight substance used widely in the pharmaceutical industry as a solvent for water-insoluble substances. However, PrG has been shown to relax respiratory smooth muscle (Black et al, 1984) and now its effect on tone of guinea-pig and rabbit trachealis muscle has been investigated further. Tracheae were removed from freshly killed New Zealand White rabbits (800-950g) and ovalbumen-sensitized Dunkin-Hartley guinea-pigs (300-500g). From each trachea two chain preparations were suspended in an organ bath containing Krebs' solution at 37°C, gassed with 5% CO_2 in O_2 (pH 7.4). Each chain was counterbalanced by 1g for isotonic recording of smooth muscle activity.

PrG was added incrementally at 5 min intervals to give concentrations of 1%, 2%, 5% and 10% v/v. Time and volume-matched control experiments utilised Krebs' solution. Tone was calculated as a percentage of the maximum contraction stimulated by carbachol (50μM) compared to maximum relaxation evoked by isoprenaline (77μM). PrG induced immediate concentration-dependent reductions in spontaneous and pharmacologically-evoked tracheal tone which were reversed readily by washing (Table I). In high concentrations (4 or 5% v/v) it also depressed significantly ($P<0.01$, n=6, Mann Whitney 'U' test) the contractile response to KCl (20-80mM).

Table 1 Tone of Guinea-pig or Rabbit Isolated Trachealis Muscle

source of tone	species	PrG concentration (v/v) 0%	1%	2%	5%	10%
Spontaneous	guinea-pig	67 (56-73)	52 (39-60)	34 (21-43)	12 (7.5-16)	5.5 (2-7.5)
0.7μM Histamine	guinea-pig	85 (79-87)	80 (77-83)	73 (64-77	37 (32-46)	19 (10-24)
0.1ml Ovalbumen	guinea-pig	83 (79-91)	74 (67-76)	53 (45-60)	28 (23-29)	14 (5.5-16)
1μM Carbachol	rabbit	88 (71-92)	73 (65-82)	39 (31-57)	2.5 (1.5-3)	0
20mM KCl	rabbit	15 (13-20)	12 (7.5-14)	6 (4.5-8)	2 (1-2.5)	0
80mM KCl	rabbit	35 (28-46)	*34 (28-44)	31 (18-36)	6.5 (2.5-8.5)	0

Values given are medians with quartiles. All reductions compared to PrG 0% v/v were significant ($P<0.05$, n=8, signed Rank Test) except *.

These results show that PrG is a powerful tracheal muscle relaxant.

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AH 21-132 SELECTIVELY INHIBITS TYPE III/IV CYCLIC NUCLEOTIDE-DEPENDENT PHOSPHODIESTERASE ACTIVITY IN BOVINE AIRWAY SMOOTH MUSCLE

Mark A. Giembycz., Judith C.W. Mak., Dieter Ukena., Edwin R. Chilvers and Peter J. Barnes. Department of Thoracic Medicine, National Heart and Lung Institute, London, SW3.

AH 21-132 (Markstein *et al*, 1984) was recently reported to produce a concentration-dependent relaxation of airway smooth muscle by a mechanism that did not involve agonism of β-adrenoceptors (Bewley & Chapman, 1988). We report here the results from studies designed to determine if AH 21-132 exerts any effect on cyclic nucleotide-dependent phosphodiesterase (PDE) and/or cyclic AMP-dependent protein kinase (A-kinase) activity.

Soluble cyclic nucleotide-dependent PDE and A-kinase activities isolated from the 25000 x g x 20 min supernatant fraction of bovine tracheal smooth muscle homogenates were estimated by the methods described by Thompson *et al* (1975) and Torphy *et al* (1982) respectively.

AH 21-132 produced a concentration-dependent relaxation of bovine tracheal smooth muscle pre-contracted with 400 nM methacholine (pD_2: 3.81 $\pm$ 0.7, n = 9) that was not antagonised by the β_2-adrenoceptor blocking drug ICI 118551 (1 µM). On a molar basis, AH 21-132 was 16000-fold less potent than isoprenaline (pD_2: 8.0 $\pm$ 0.5, n = 9) but produced the same degree of relaxation. AH 21-132 effectively inhibited cyclic AMP hydrolysis in a concentration-dependent manner (Table I). In relation to other PDE inhibitors, AH 21-132 was less potent than zaprinast, equipotent with 3-isobutyl-1-methylxanthine (IBMX) and much more potent than both SKF 94120 and rolipram (Table I). Separation of PDE isoenzymes over QAE-sepharose resolved three peaks of hydrolytic activity when the assay was performed in the presence of 2 mM EGTA. Peaks I and II selectively hydrolysed cyclic GMP and corresponded to the Type Ia and Type II PDE isoenzymes respectively. In contrast, the activity associated with peak III displayed selectivity for cyclic AMP hydrolysis and was probably a mixture of the Type III and Type IV PDE isoenzymes. AH 21-132 was *ca* 70-fold more potent at inhibiting the activity of the Type III/IV PDE isoenzymes than of the Type Ia (Table I).

Table I. Effect of AH 21-132 and other Phosphodiesterase Inhibitors on Cyclic AMP Hydrolysis

DRUG	IC_{50} (µM)	PDE ISOENZYMES	IC_{50} (µM)
AH 21-132	42.6 $\pm$ 4.5 (8)	Type Ia (Cyclic GMP)	126.1 (3)
Zaprinast	9.2 $\pm$ 2.0 (4)	Type II (Cyclic GMP)	60.2 (3)
IBMX	31.4 $\pm$ 9.4 (6)	Type III/IV (Cyclic AMP)	2.1 (3)
SKF 94120	557.9 $\pm$ 149.6 (6)		
Rolipram	453.9 $\pm$ 237.1 (4)		

Consistent with the PDE data, AH 21-132 produced concentration-dependent increases in the percentage of A-kinase in the active state (from 31.4 $\pm$ 2.9% at rest to 52.5 $\pm$ 2.7% after 15 min challenge with 1 mM AH 21-132; n = 8). The activation of A-kinase was not antagonised by ICI 118551 (1 µM).

We conclude that the relaxant mechanism of action of AH 21-132 in bovine airway smooth muscle is due, at least in part, to the accumulation of cyclic AMP and subsequent activation of A-kinase. These effects are likely to be due to the ability of AH 21-132 to inhibit cyclic AMP hydrolysis catalysed by one or more of the PDE isoenzymes associated with the peak III activity.

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INHIBITION OF THE PHOSPHOINOSITIDE RESPONSE TO 5-HT IN BOVINE TRACHEAL SMOOTH MUSCLE BY AGENTS WHICH INCREASE CYCLIC AMP LEVELS.

I.P. Hall & S.J. Hill, Department of Physiology & Pharmacology, Queen's Medical Centre, Nottingham NG7 2UH.

Contraction of airway smooth muscle in response to histamine, carbachol and 5-hydroxytryptamine (5-HT) is thought to involve hydrolysis of phosphatidylinositol-4,5-bisphosphate with the consequent formation of the intracellular second messengers inositol-1,4,5-trisphosphate and diacyglycerol (Hashimoto et al., 1985). We have previously described how agents which elevate cyclic AMP in bovine tracheal smooth muscle inhibit the formation of ^{3}H-inositol phosphates elicited by histamine (Hall & Hill, 1988; Hall et al., 1988). We report here that 5-HT induces concentration related ^{3}H-inositol phosphate formation in this tissue and that this response can also be inhibited by agents which elevate tissue cyclic AMP levels.

Formation of ^{3}H-inositol phosphates was measured in the presence of 5mM lithium chloride in slices of bovine tracheal smooth muscle which had been prelabelled with ^{3}H-myo-inositol as previously described (Hall & Hill, 1988). Where used phosphodiesterase inhibitors were added 20 min before 5-HT. All other agents were added simultaneously with 5-HT and incubation with agonists was continued for 45 min.

5-HT produced concentration related ^{3}H-inositol phosphate formation with an EC_{50} of 41 $\pm$ 18 μM (n=4) in bovine tracheal smooth muscle. The response to 100 μM 5-HT was inhibited by 50 nM ketanserin (88 $\pm$ 7 % inhibition, n=3). 100μM 5-HT increased the incorporation of ^{3}H-myo-inositol into the tissue phospholipids to 175 $\pm$ 27 % (n=6) of control (=100%) levels. The effect of a variety of agents capable of elevating cyclic AMP content in bovine tracheal smooth muscle (see Hall et al., 1988) on the ^{3}H-inositol phosphate response to 100μM 5-HT was examined. Salbutamol (1μM, 53 $\pm$ 5 % inhibition, n=11, $p < 0.05$), forskolin (1μM, 52 $\pm$ 8% inhibition, n=4, $p < 0.05$), 3-isobutyl-1-methylxanthine (IBMX) (1mM, 85 $\pm$ 3% inhibition, n=8, $p < 0.05$) and rolipram (100 μM, 89 $\pm$ 4 % inhibition, n=4, $p < 0.05$) all inhibited the ^{3}H-inositol phosphate response to 100 μM 5-HT. In addition, a smaller inhibition (30 $\pm$ 2%) was elicited by the membrane permeant cyclic AMP analogue 8-brom-cyclic AMP (n=3).

These results suggest that, as with histamine, the ^{3}H-inositol phosphate response to 5-HT in bovine tracheal smooth muscle can be inhibited by agents capable of elevating tissue cyclic AMP content through a variety of different mechanisms.

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COMPARISON OF THE USE OF HISTONE AND KEMPTIDE IN DETERMINING A-KINASE ACTIVITY IN GUINEA-PIG TRACHEALIS.

John M. Langlands & Ian W. Rodger[1], Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, Canada and [1]Department of Physiology and Pharmacology, University of Strathclyde, Glasgow, U.K.

We have previously reported that determination of the cyclic AMP-dependent protein kinase (A-kinase) activity ratio in guinea-pig trachealis gives a much clearer indication of changes in cAMP content than measurement of cAMP itself (Langlands et al, 1987). Here we report some differences in the results obtained, for cytosolic A-kinase activity in guinea-pig trachealis, when using two different substrates, histone IIa and kemptide.

Cytosolic A-kinase activity was measured in the absence and presence of added cAMP (10μM) using a modification of the original method described by Corbin and Reimann (1974) and the activity ratio calculated. Results are expressed as the mean ± S.E. mean of at least 5 experiments.

Using histone, increasing concentrations of Na^+ (0-400mM) in the reaction medium produced a concentration-dependent inhibition of the enzymic activity (from 0.51±0.04 to 0.20±0.04nmol PO_4/min/mg protein) in the presence of added cAMP but not in the absence of cAMP. This resulted in an increase in the enzyme activity activity ratio (from 0.19±0.01 to 0.67±0.08). Thus, using histone an overestimation of the activity ratio occurs in high [Na^+]. In contrast, phosphorylation of kemptide was not inhibited by Na^+ (up to 400mM) in either the absence or presence of added cAMP.

Increasing [Na^+] (from 6-400mM) in the homogenising buffer produced a concentration-dependent elevation in the basal A-kinase (-cAMP) activity (from 0.20±0.02 to 0.40±0.09nmol PO_4/min/mg protein). Such a change caused the activity ratio to increase (from 0.20±0.01 to 0.31±0.01). Thus, using kemptide low [Na^+] led to an underestimation of the activity ratio.

Addition of a protein kinase inhibitor (PKI) to the reaction buffers containing histone inhibited A-kinase activity in a concentration-dependent manner, producing a maximum inhibition of 40% of basal (-cAMP) and 80% of stimulated (+cAMP) enzyme activity. Thus, the PKI reduced the activity ratio (from 0.30±0.08 to 0.18±0.04). In contrast, using kemptide, the PKI abolished all phosphotransferase activity both in the absence and in the presence of added cAMP. Thus, only when using histone is it necessary to use a PKI to determine the proportion of phosphotransferase activity attributable to cAMP-independent processes. Basal and stimulated A-kinase activity of trachealis, corrected for cAMP-independent phosphotransferase activity, was 0.72±0.033 and 2.35±0.34nmol PO_4/min/mg protein using kemptide but only 0.17±0.005 and 0.65±0.04nmol PO_4/min/mg protein using histone.

The results support the preferential use of kemptide over histone in determining cytosolic A-kinase activity in guinea-pig trachealis since (i) cAMP-independent phosphotransferase activity is not expressed, (ii) it is a better substrate for A-kinase and (iii) physiologically relevant [Na^+] can be used to improve the yield of catalytic subunits without inhibiting substrate phosphorylation.

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EVIDENCE THAT $GABA_B$ RECEPTORS CAN MODULATE RELEASE OF SUBSTANCE P FROM CAPSAICIN-SENSITIVE NEURONS IN RAT TRACHEA

N.J. Ray*, A.J. Jones & P. Keen, Department of Pharmacology, University of Bristol, The Medical School, University Walk, Bristol BS8 1TD.

In rat trachea, changes in vascular permeability take place upon antidromic activation of capsaicin-sensitive sensory neurons *in vivo* (Lundberg & Saria, 1982). Substance P has been proposed as a candidate mediator for these vascular responses and it may play a role in respiratory disease, notably asthma (Barnes, 1986). It has been suggested that GABA receptor activation decreases the contractile response of guinea-pig trachea to cholinergic nerve stimulation *in vitro* (Tamaoki et al., 1987). We have developed a multi-superfusion system to study the control of the release of Substance P-like immunoreactivity (SP-LI) from rat trachea and have investigated the effect of γ-aminobutyric acid (GABA) receptor activation on potassium stimulated SP-LI release.

Eight spirally cut trachea from male rats (Wistar 350-400g) were mounted in parallel oxygenated glass chambers and superfused with Krebs' solution (pH 7.4, 37°C gassed with 95% O_2/5% CO_2) at 5 ml/min to allow equilibration. After 60 min. phosphoramidon (1 μM), captopril (100 μM) and bacitracin (20 mg/l) were added to the Krebs' solution and the flow rate adjusted to 1 ml/min. After a further 15 min. four fractions of 5 min. from each trachea were simultaneously collected in vials containing a final concentration of 0.1% trifluoracetic acid. Fractions were then concentrated on Sep-Pak C_{18} cartridges, lyophilised and reconstituted in barbitone buffer (pH 8.6) for radioimmunoassay (RIA) of SP-LI. Drugs were added to the superfusion fluid reservoir for 4 min. during the third fraction. Changes in SP-LI release were calculated as the difference in fractional release constants between the mean of fractions 1 and 2 and the mean of fractions 3 and 4 based on the recovery of exogenous SP (40 pg/ml) determined as 62.2 ± 3.2% (n=12). None of the agents used interfered with the RIA.

Capsaicin (50 mg/kg s.c.), administered neonatally, caused a 93.2 ± 6.4% ($p < 0.001$, n=8) reduction in tracheal SP-LI content suggesting that the great majority of tracheal SP-LI is neuronal in origin.

Capsaicin (1-500 μM), veratridine (10-50 μM), bradykinin (1-10 μM), and isosmolar high potassium (K^+) solution (60 mM) significantly increased SP-LI release above spontaneous release values. Removal of calcium ions resulted in total abolition of 60 mM K^+ stimulated release of SP-LI ($p < 0.001$, n=8).

GABA (1-100 μM) and (±)baclofen (1-100 μM) did not affect spontaneous release but, when added 1 min. prior to, and during exposure to 60 mM K^+, produced dose related inhibition of SP-LI release with maximum decreases of 77.7 ± 18.8% ($p < 0.001$, n=8) and 52.9 ± 20.2% ($p < 0.01$, n=12) respectively.

This technique may prove a powerful tool in the investigation of the control of airway sensory neurones. Furthermore, GABA, via $GABA_B$ receptors may play a role in the physiological regulation of airway function with widespread pharmacological implications.

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NEUROGENIC CONTRACTIONS OF DOG ISOLATED BASILAR ARTERY: INVOLVEMENT OF A THROMBOXANE-LIKE SUBSTANCE

LA Edwards, HE Connor and W Feniuk, Department of Cardiovascular Pharmacology, Glaxo Group Research Ltd., Ware, Herts. SG12 0DP,

Electrical field stimulation of isolated cerebral arteries produces a contraction mediated via sympathetic nerves which is not susceptible to blockade by α-adrenoceptor antagonists (Lee et al., 1976). In the present study, we have investigated the effects of a variety of receptor blocking drugs on neurogenically mediated contractions of dog isolated basilar artery, obtained from beagle dogs killed with pentobarbitone sodium (100mg/kg i.v.). Arteries were cut into ring segments (3-4mm in length) and suspended in modified Krebs solution (Apperley et al., 1976) at a final resting tension of 1g. Isometric tension was recorded. Segments were positioned between 2 platinum wire electrodes and stimulated every 10 mins (0.1msec pulse width, 15-20v and 80 pulses, delivered at 2, 4 and 8Hz). These parameters were maximal for eliciting reproducible, frequency-dependent contractions which were abolished by tetrodotoxin (1μM). The responses were not modified by phentolamine (1μM), atropine (1μM), methiothepin (0.1μM) or ketanserin (1μM). However, responses were markedly reduced by the thromboxane receptor antagonists GR32191 (1-10nM; Lumley et al., 1987) (Figure 1) or BM 13.177 (0.1 - 10μM; Stegmeier et al., 1984) or by the cyclo-oxygenase inhibitor flurbiprofen (0.5μM). In dog basilar artery segments which had been previously perfused with Triton X-100 (0.1%, 1ml/min for 1 min) to remove the endothelium (Connor and Feniuk, 1989), electrical stimulation still elicited neurogenic contractions which were blocked by GR32191 (1-10nM). This effect of GR32191 was specific since GR32191 (0.1-1μM) did not reduce the contraction produced by potassium chloride (30mM). Furthermore, in rabbit isolated ear artery, neurogenic responses to electrical field stimulation were not modified by GR32191 (0.01-1μM).

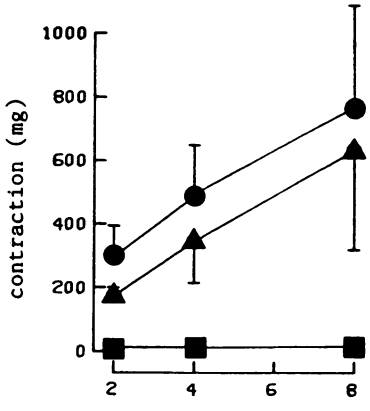

Figure 1 Neurogenic responses to electrical stimulation of dog basilar artery before (●) and in the presence of 1nM (▲) and 10nM (■) GR32191. Values are means ± S.E.M. from n = 5 experiments

These results suggest that, in dog isolated basilar artery, neurogenic contractions are not mediated via noradrenaline or 5-hydroxytryptamine release, but appear to be mediated via the release of a thromboxane-like substance. The source of this agent does not appear to be the endothelium nor is its release a consequence of contraction of the preparation. However, in view of a recent report (Trachte et al., 1988) on the ability of nerves to synthesise thromboxane, one explanation for our finding is that the thromboxane-like substance is neuronally derived.

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POTENTIATING ACTION OF 5-HYDROXYTRYPTAMINE (5-HT) ON ELECTRICALLY-INDUCED CONTRACTIONS IN THE MOUSE URINARY BLADDER.

A Cleal[1], M Corsi, W Feniuk[1], G Gaviraghi, G Toson, D Trezise[1], D Trist, Dept. of Pharmacology, Glaxo-Duncan Research Laboratories, Verona and [1] Dept. of Cardiovascular Pharmacology, Glaxo Group Research Ltd., Ware, Herts.

In mouse isolated urinary bladder, 5-HT potentiates the contractile response to electrical stimulation (ES), probably through the activation of the 5-HT_{1B} receptors (Holt et al., 1986). We have further analyzed this potentiating action using a variety of 5-HT agonists and antagonists.

Mouse urinary bladders were mounted in organ baths for isometric tension recording, immersed in Krebs solution containing atropine (1 uM) and electrically stimulated by platinum electrodes (10 Hz for 2s, 0.1 ms pulse width, supramaximal voltage, every 60s). Cumulative concentration-response curves to 5-HT agonists were constructed until a maximal response was obtained. Where the effect of antagonists were examined, these were added to the bath two hours before 5-HT agonists.

5-HT agonists caused a concentration-dependent increase in the contractile response to ES with a rank order of agonist potencies of 5-carboxamidotryptamine (5-CT)$>$ 5-HT$>$ α-methyl 5-HT$>$ GR 43175 (Humphrey et al., 1988) $>$ 2-methyl 5-HT. The potentiating action of 5-CT was biphasic and the slope (0.75 ± 0.03) of the concentration response to 5-HT was significantly ($P < 0.01$) less than unity.
Taken together these findings suggest that more than one receptor is activated by 5-HT; probably 5-HT_1-like and 5-HT_2 receptors. To further analyse this hypothesis, we have examined the effects of 5-HT antagonists against the selective 5-HT_1-like agonist "5-CT" and the selective 5-HT_2 agonist "α-methyl 5-HT". The lower part of the concentration-response curve to 5-CT (0.0001-1uM) was shifted to the right by cyanopindolol (0. 003-0.1uM) and methiothepin (0.01-3uM) with pA_2 values (95% C.L.) of 8.8 (8.4-10.0) and 8.0 (7.6-8.9) respectively. Schild analyses gave slopes not significantly different from unity ($P < 0.05$). Ketanserin (0.1-1 uM) and GR 38032F (1 uM) were ineffective.
The concentration-response curve to α-methyl 5-HT was shifted to the right in a seemingly parallel fashion by ketanserin (0.003-0.03 uM) with an "apparent" pA_2 of 8.5. However the Schild plot slope parameter was significantly less than unity (0.54 ± 0.36) and a higher concentration (0.1 uM) clearly reduced the maximal response. Methiothepin (0.003-0.1 uM) caused parallel displacement to the right but produced a similar depression of the maximum at all concentrations. A Schild plot constructed from these displacements had a slope of 0.86 ± 0.29 (s.e., 15 d.f.). The pA_2 was estimated as 8.5 ± 0.3 (95% C.L.) with the slope restrained to unity. GR 38032 F(1 uM) and cyanopindolol (1 uM) were ineffective.
Contractile responses to direct electrical stimulation (6 ms, supramaximal voltage, 10 Hz for 2s) in the presence of TTX (1 uM) were not enhanced by 5-HT up to a concentration of 10uM. Furthermore, 5-HT did not modify contractile responses to submaximal concentrations of acetylcholine (10-30 uM) and concentration response curves to carbachol (0.01 - 30 uM).

These results suggest that 5-HT potentiates the contractile response to ES in the mouse urinary bladder through the activation of 2 receptor subtypes: the 5-HT_{1B} (already identified by Holt et al., 1986) and the 5-HT_2. The location of these receptors appears to be pre-synaptic.

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CALCIUM AND CONTRACTILITY OF HUMAN COLONIC SMOOTH MUSCLE

D.J. Butler & K. Hillier, Clinical Pharmacology Group, Medical Faculty, University of Southampton, Southampton SO9 3TU, England.

The fundamental role of calcium (Ca^{2+}) as activator of contractile activity in smooth muscle has been widely established. The importance of extracellular or intracellular sites for Ca^{2+} mobilisation depends upon the tissues and stimulant. We have studied the behaviour of selected Ca^{2+} channel antagonists and sources of activator Ca^{2+} in human colonic smooth muscle stimulated by KCl, ACh or BAY K 8644.

Macroscopically normal large bowel was obtained from patients undergoing colonic resection for carcinoma. Longitudinal or circular intertaenial muscle strips were suspended in aerated Krebs under 1g initial tension and with 2h equilibration. KCl (20-120mM) induced slow-developing contractile activity with peak phasic followed by sustained tonic response components. Contracture was abolished in Ca^{2+} free/EGTA Krebs. Nitrendipine, verapamil and diltiazem (10^{-10}-10^{-6}M) caused concentration-dependent inhibition with no significant discrimination between phasic and tonic KCl response components. This contrasts with a relatively greater effect of Ca^{2+} antagonists on the tonic component in guinea-pig colon (not shown). Nitrendipine was most persistent in its effect with little tissue recovery after 2h, despite frequent washing; inhibition by verapamil or diltiazem was readily reversible. Calmodulin antagonists TMB8 and W7 (10^{-5}-10^{-3}M) also reduced KCl-stimulated responses.

	IC50 values (mean ± SEM) - 60mM K^+ n=4-10	
	Phasic	Tonic
Nitrendipine	4.0×10^{-8} M (± 4.4×10^{-8})	4.2×10^{-8} M (± 3.2×10^{-8})
Verapamil	3.0×10^{-7} M (± 2.3×10^{-7})	4.1×10^{-7} M (± 3.8×10^{-7})
Diltiazem	3.7×10^{-7} M (± 3.5×10^{-7})	2.9×10^{-7} M (± 2.4×10^{-7})
TMB8	2.7×10^{-5} M (± 1.7×10^{-5})	4.3×10^{-5} M (± 1.9×10^{-5})
W7	1.5×10^{-5} M (± 1.4×10^{-4})	3.5×10^{-4} M (± 2.1×10^{-4})

Bay K 8644 (10^{-8}-10^{-6}M) induced predominantly concentration-dependent rapid spike activity but at > 5×10^{-6}M there was no stimulatory effect. Nitrendipine, verapamil and diltiazem (10^{-7}-10^{-5}M) inhibited Bay K 8644 contracture suggesting similar or closely linked drug-receptor sites. ACh rapidly stimulated colonic tissue, inducing recurrent spike activity on an elevated hypertonic baseline. Ca^{2+} antagonists (10^{-8}-5×10^{-6}M) selectively abolished recurrent spiking without reducing sustained tone.

[^{45}Ca] efflux was studied in 20mg muscle pieces preloaded for 1h in [^{45}Ca]-containing Krebs (10μCi ml^{-1}). Perfusion at 2.5ml min^{-1} with non-isotopic Krebs resulted in biphasic efflux, with fast extracellular washout followed by slower removal of more tightly bound intracellular Ca^{2+} ions. KCl (60-100mM) stimulated the rate coefficient for slow Ca^{2+} efflux and this was abolished with Ca^{2+}-free/EGTA or 20mM La^{3+} Krebs as perfusates. Nitrendipine, verapamil and diltiazem at concentrations that inhibited KCl-induced tension development, did not affect KCl-stimulated slow Ca^{2+} efflux. Thus contraction of human colonic muscle requires Ca^{2+} from both extracellular and intracellular sources, the contribution from each depending on stimulus and phase of mechanical activity studied. Extracellular Ca^{2+} influx is predominantly via voltage-dependent Ca^{2+} channels.

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EFFECT OF PAPAVERINE ON KCL-INDUCED CONTRACTIONS IN RAT ISOLATED SEMINAL VESICLE.

E. Greenidge[1] and F.A. Wali[2]. [1] Department of Physiology, The London Hospital Medical College, Whitechapel, London E1, and [2] Respiratory and Anaesthetics Unit, The Hospital for Sick Children, Great Ormond Street, London WC1N, U.K.

Papaverine produces relaxation of smooth muscle and reduces the contractile response to excitatory agents (Bolton,1979; Brading,Burdyga & Scripnyuk,1983). However,the mechanism of action of papaverine still remains unclear.Possible mechanisms include inhibition of phosphodiesterase activity in various smooth muscle (Pöch & Kukovetz,1971; Miyamoto,Takayanagi,Ohkubo & Takagi,1976) and/or blockade of calcium influx (Andersson,Andersson,Hender & Persson,1977).In addition,Tsuda, Urakawa & Saito (1977) have suggested that the inhibition,by papaverine,of tonic tension,induced by high K^+ in guinea-pig taenia coli,was due to its inhibition of mitochondrial respiration. The purpose of the present investigation was to see if papaverine reduced the KCL contraction,in rat isolated seminal vesicle,and to analyse further the possible mechanism of action in this preparation.

Seminal vesicles,from male,Sprague-Dawley rats,weighing 200-300g, were used.The preparation was cut spirally,into strips,2-3 cm long and 2 mm wide, and was set up in an organ bath containing 20 ml Krebs-Henseleit solution maintained at 38±2°C and bubbled with 5% CO_2 in O_2. The contractile responses produced by KCL were recorded isometrically.

KCL (1-5x10^2 mM) produced concentration-dependent contractions in rat seminal vesicle, the response being mainly biphasic; a fast phasic followed by a slow and prolonged tonic contraction. A mean maximum tonic contraction of 1.6±0.2 g(mean± s.e.,n=6) was obtained with 150 mM KCL. The mean concentration which produced 50% maximum contraction was 54±2.1 mM. Papaverine,itself,had little effect on contractility of seminal vesicle, but significantly reduced KCl tonic contraction.The mean concentration of papaverine that produced 50% maximum reduction of KCL tonic tension was 212±15 µM.In the absence of papaverine,the KCL contraction continued to rise,slowly but steadily,until a maximum plateau had reached,in 10-15 min.The results are shown in Table 1.

Table 1. Contractile responses of KCL and the effect of papaverine on rat isolated seminal vesicle.

(µ M) Papaverine	KCL with Papaverine (1) (g) tension	KCL without Papaverine (2) (g) tension	[(2-1)/1 x 100] % Change	P <
0.27	1.5±0.1	1.6±0.2	7%	N.S.
2.7	1.1±0.09	1.6±0.1	27%	0.05
27	0.9±0.07	1.7±0.3	89%	0.001
270	0.6±0.06	1.7±0.4	183%	0.001
27 x 10^2	0.55±0.05	1.75±0.5	218%	0.001
27 x 10^3	0.50±0.03	1.85±0.4	270%	0.001
27 x 10^4	0.50±0.02	1.95±0.5	290%	0.001

The results are means±s.e.,n=6. Papaverine reduced KCL (54 mM) tonic tension,and it is possible that the relaxation observed may be due to blockade of slow Ca^{2+} channels(Brading,et.al.,1983),responsible for generation of the tonic plateau tension in this preparation.

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OCCURRENCE AND SOME PROPERTIES OF AMINE OXIDASES IN HUMAN COLON

C.J. Barwell, C.A. Canham, P. Bull and P.M. Perry[1], Pharmacy and Biomedical Sciences, Portsmouth Polytechnic, Portsmouth PO1 2DZ and [1]Queen Alexandra Hospital, Portsmouth, PO6 3LY.

Monoamine oxidases (MAO) and semicarbazide-sensitive amine oxidases (SSAO) occur in mammalian tissues and may be distinguished by their inhibitor sensitivity. MAO-A is inhibited selectively by clorgyline, MAO-B by deprenyl (Fowler et al., 1981) and SSAO by semicarbazide (Callingham & Barrand, 1987). Activities of MAO and SSAO have been reported in mammalian small intestine (Barwell & Canham, 1988) and SSAO in colon (Lewinsohn et al., 1978). However, there is little data on relative activities of MAO and SSAO in human colon, which was the subject of this investigation. Apparently normal ascending colon was obtained from female patients undergoing surgery for colonic carcinoma and separated, by stripping, into mucosal and serosal components. Homogenates were prepared and amine oxidase activity assayed with 5 mM tyramine (TA) and benzylamine (BZ) under conditions described previously (Barwell & Canham, 1988).

Mucosal and serosal homogenates contained TA and BZ deaminating activity and Michaelis constants were determined as 210 μM for TA and 320 μM for BZ. Deprenyl at 10^{-3}M inhibited completely TA deamination with no significant effect upon BZ deamination. In contrast, semicarbazide at 10^{-3}M inhibited completely BZ deamination with no significant effect upon TA deamination. Table 1 shows that activity was highest, in both mucosal and serosal homogenates, with BZ and that the TA and BZ deaminating activity of serosal was higher than mucosal homogenates. Titration of serosal TA deaminating activity with clorgyline and deprenyl gave monophasic inhibition curves and clorgyline was the more potent inhibitor.

Table 1 Tyramine and benzylamine deaminating activity in homogenates of human colon mucosal and serosal tissue

Colon sample	Deaminating activity (nmol g wet wt^{-1} h^{-1})			
	mucosal tissue		serosal tissue	
	TA	BZ	TA	BZ
A	220 ± 30	2700 ± 130	2200 ± 260	10900 ± 950
B	50 ± 25	2900 ± 360	1360 ± 120	5488 ± 460

Values are mean ± s.e., n = 3. Tyramine (TA) and benzylamine (BZ) 5 mM.

Results with selective inhibitors indicated that TA deamination was due to MAO-A and BZ to SSAO. The occurrence of MAO-A as the predominant form of MAO was similar to rat small intestine (Barwell & Canham, 1988) but the low MAO-A activity of mucosal tissue was unexpected. Others (Lewinsohn et al., 1978) found that MAO-B constituted one quarter of the BZ deaminating activity of human colon but it was not detectable in these tissue samples. The low mucosal MAO-A activity and apparent absence of MAO-B may be associated with colonic carcinoma and/or its treatment.

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IMMUNOGENICITY AND HEPATOTOXICITY OF AMODIAQUINE IN THE RAT

J.B. Clarke, J.L. Maggs, N.R. Kitteringham and B.K. Park. Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

Amodiaquine (AQ; Camoquine) is an anti-malarial that has proved effective against chloroquine resistant *Plasmodium falciparum*, but has been associated with hepatotoxicity and agranulocytosis in man (Neftel *et al.*, 1986). Both cytotoxic and immune-mediated mechanisms have been implicated. In this study we have examined the effect of dose and route of administration on the immunogenicity of AQ in a rat model and concurrently measured perturbation of the white blood cell (w.b.c.) population and liver function. Six groups of male Wistar rats (200 - 300g, n = 5) were used. The first four groups were given AQ (in PEG 200, 1ml/kg for 4 days, i.m.) at doses of 2.15, 21.5, 268 and 538 μmol/kg. Two further groups were given AQ (268 μmol/kg) either i.p. or p.o. Blood samples were taken prior to and at several times points following immunisation and peripheral w.b.c. counts measured. Serum transaminase (ALT) levels were measured by the method of Reitman and Frankel (1957) and specific anti-AQ antibodies determined by ELISA (Christie *et al.*, 1989). AQ proved to be immunogenic in some or all of the rats in each group regardless of route of administration. It was possible to show by hapten-inhibition (Christie *et al.*, 1989) specific anti-AQ antibodies in each individual animal given AQ i.m. (268 or 538 μmol/kg).

Route	Dose (μmol/kg)	Mean antibody titre
i.m.	2.15	71 ± 32
i.m.	21.5	44 ± 17
i.m.	268	352 ± 221
i.m.	538	490 ± 544
i.p.	268	1349 ± 939
p.o.	268	91 ± 54

The w.b.c. count and serum ALT levels were unaffected at 268 μmol/kg, but at the highest dose (538 μmol/kg), immmediately after the last injection of AQ, the w.b.c. count was significantly reduced and serum ALT levels slightly but significantly raised ($P < 0.005$) from a mean of 12.7 to 45.7 SF units. Thus AQ induced specific antibody formation at doses that did not perturb liver function or the w.b.c. population in a rat model.

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THE "IN VITRO" EFFECTS OF NIFEDIPINE, DILTIAZEM, VERAPAMIL AND ENOXACIN ON HEPATIC MICROSOMAL DRUG METABOLISING ENZYMES.

Barclay CG, Meredith PA., University Department of Materia Medica, Stobhill General Hospital, Glasgow G21 3UW.

Although verapamil, nifedipine and diltiazem are all calcium channel blocking drugs they are diverse in structure and pharmacology. Along with enoxacin, a quinolone antibacterial agent, all three calcium antagonists have been implicated in drug interactions (Echizen et al 1986; Beckmann et al 1987) with a variety of drugs particularly theophylline. A number of these drugs also show a decrease in apparent oral clearance in translation from acute to steady state treatment and this, in the case of verapamil, has been attributed to decreases in hepatic enzyme activity. This study was designed to evaluate the "in vitro" effects of nifedipine, diltiazem, verapamil and enoxacin on microsomal drug oxidising enzymes.

Pooled hepatic microsomal fractions were prepared from the livers of male Wistar rats. The activity of the drug metabolising enzymes were determined in the microsome suspensions by standard spectrophotometric and spectrofluorometric techniques and the enzyme assays included aminopyrene N-demethylase, aryl hydroxylase, p nitroanisole O-demethylation, 7 ethoxy-coumarin O-deethylase and aniline hydroxylase. Various concentrations (0.05-5.0 umoles) of the four drugs were added to the assay of the enzymes and activity was expressed as percentage of the enzyme activity in the absence of drug. The relationship between enzyme activity and added drug concentration was examined using the Hill equation constraining the maximum inhibition to be 100%. From this approach the IC_{50} values were derived, that is the concentration of drug producing 50% enzyme inhibition. Six replicate inhibition curves were constructed for each drug and the different IC_{50} values for each were compared by analysis of variance.

The IC_{50} values for the drug added to the various enzymes are shown below:

	IC_{50} (umoles)				
	1	2	3	4	5
Enoxacin	2.6±0.1	0.34±0.05	>5	0.7±0.2	1.0±0.1
Nifedipine	3.2±0.5	3.8±0.4	3.9±0.6	2.6±0.2	3.9±0.6
Verapamil	0.31±0.04	0.7±0.1	3.8±0.6	0.8±0.08	1.6±0.2
Diltiazem	0.34±0.06	0.19±0.02	2.4±0.2	1.3±0.11	0.4±0.08

1 = 7 ethoxycoumarin deethylase 2 = aryl hydroxylase 3 = aminopyrene demethylase 4 = p nitroanisole 0 - demethylase 5 = aniline hydroxylase.

It is apparent from this table that for all enzymes nifedipine is a very weak inhibitor. In contrast the other three drugs are selective inhibitors of drug metabolising enzymes. In particular, enoxacin is a potent inhibitor of ar yl hydroxylase while having little or no effect on aminopyrene demethylase. Verapamil and diltiazem are significantly more potent inhibitors than nifedipine and enoxacin of all the enzymes studied except aminopyrene demethylase.

It is apparent from this "in vitro" study that nifedipine is only a very weak inhibitor of drug metabolising enzymes. In contrast enoxacin, verapamil and diltiazem are all selective inhibitors of some drug metabolic enzymes but not others. This suggests these drugs all have the potential for significant drug interactions in man but that these interactions may not be readily predicted.

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THE EFFECTS OF CASTRATION AND TAIL DOCKING ON PLASMA CATECHOLAMINES IN LAMBS

S.J. Ley, A. Livingston & A.E. Waterman, Departments of Pharmacology and Veterinary Surgery, University of Bristol, Bristol BS8 1TD.

A study was conducted to examine the effects of the acute pain resulting from castration and tail docking in lambs on the plasma levels of noradrenaline (NA) and adrenaline (Ad).

A group of seven Clun cross lambs were used in the study. Lambs were brought into the laboratory with their dams, in large, wheeled crates. They were removed during blood sampling, castration and docking, and returned immediately afterwards.

On day 4 after birth, the male lambs were castrated. This was done by cutting open the scrotum and pulling out both testes. Blood samples were taken before and immediately after surgery and at 30, 60 and 120 minutes. Blood samples were also taken from a female sibling on day 4. On day 6 the male and female lambs were tail-docked. This is a standard farming technique done by the application of a tight rubber ring around the tail about 5 cm below the anus.

Plasma catecholamines were measured by hplc with electrochemical detection (Hjemdahl et al., 1979). The effects of castration and tail docking on the plasma adrenaline and noradrenaline concentrations are shown in Tables 1 and 2. The pre-surgery levels were not different from those of the appropriate female siblings and neither the NA nor the Ad were changed by castration. In the female lambs no changes in NA or Ad were observed over 2h after tail-docking. The male lambs showed no change in plasma adrenaline. However, 60 minutes after tail docking, the plasma NA concentration was significantly elevated in male lambs.

The data would suggest that castration on day 4 had sensitised the male lambs, leading to an exaggerated response to tail docking two days later.

Table 1. Plasma Noradrenaline (nM)

	Tail Docking		Castration	
	♂ n=4	♀ n=3	♂ n=3	♀ n=3
Pre-surgery	13.1 ± 2.3	10.0 ± 2.1	17.2 ± 0.4	16.5 ± 3.3
Post-surgery	14.2 ± 3.3	13.3 ± 2.5	16.7 ± 1.2	
+ 30 min	14.4 ± 3.0	15.0 ± 4.0	18.6 ± 1.1	
+ 60 min	20.4 ± 2.3 ***	12.5 ± 3.3	16.7 ± 0.4	
+ 120 min	12.4 ± 2.3	10.4 ± 2.2	15.6 ± 2.3	

*** $p < 0.001$ Paired t-test

Table 2. Plasma Adrenaline (nM)

	Tail Docking		Castration	
	♂ n=4	♀ n=3	♂ n=3	♀ n=3
Pre-surgery	4.5 ± 1.0	3.7 ± 0.7	4.8 ± 1.2	5.3 ± 1.2
Post-surgery	4.4 ± 0.3	4.3 ± 1.0	4.9 ± 1.0	
+ 30 min	6.1 ± 1.3	4.8 ± 1.6	6.2 ± 1.0	
+ 60 min	6.8 ± 2.5	4.9 ± 1.7	5.0 ± 1.2	
+ 120 min	4.5 ± 0.7	2.8 ± 0.2	5.2 ± 0.6	

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BEHAVIOURAL AND BIOCHEMICAL EFFECTS OF CHRONIC INTRATHECAL INFUSION OF TRH ANTIBODIES.

D. Connor, K.C.F. Fone & G.W. Bennett, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Chronic immunization of endogenous thyrotrophin-releasing hormone (TRH) in brain by intracerebral administration of purified TRH antibodies enhances the behavioural responses to pentobarbitone and a TRH analogue (Lighton et al., 1986) and attenuates the hyperactivity response to intraccumbens histamine (Bristow & Bennett, 1988) without altering the pituitary-thyroid axis. Previous studies have shown that intrathecal TRH analogue infusion produces wet dog shakes (WDS) and alters ventral spinal cord 5-HT (Fone et al., 1988). In the present study, TRH antibodies were chronically infused intrathecally in rats to determine the effects of spinal immunization of this peptide on TRH- and 5-hydroxytryptamine (5-HT)-induced motor behaviours and spinal cord biochemical parameters.

Male Wistar rats (250-350g) were cannulated intrathecally (i.t.) along the dorsal thoraco-lumbar sub-arachnoid space as described previously (Fone et al., 1988) and after 7 days recovery osmotic minipumps (Alzet) containing either purified TRH antibodies (TRH-Ab), control serum or saline were implanted intrascapularly under sodium methohexitone anaesthesia (60 mg/kg i.p.) and connected to the i.t. cannulae. Sera IgG fractions were extracted with saturated ammonium sulphate, filtered, dialysed, shown to specifically bind TRH and diluted 1:10 with 0.9% sterile saline. Back muscle contractions (BMC) and WDS were counted for 25 min following i.t. injection of the 5-HT agonist, 5-methoxy-N,N-dimethyltryptamine (5-MeODMT, 20 µg, day 13) and the TRH analogue (pGlu-His-3,3'-dimethyl-ProNH$_2$, RX 77368, 1 µg, day 14) while temporarily disconnecting the minipumps. The thoraco-lumbar spinal cord and brain stem were then taken (day 14) to determine TRH, substance P and calcitonin gene-related peptide (CGRP) levels using specific radioimmunoassays, 5-HT and its major metabolite, 5-hydroxyindoleacetic acid (5-HIAA) by HPLC with electrochemical detection, and choline acetyltransferase (ChAT) activity by a radioenzymatic assay (Fone et al, 1988).

RX 77368-induced WDS were significantly reduced during the first 9 min of the response following TRH-Ab infusion (6 $\pm$ 3, mean $\pm$ s.e.m., n=7) compared with either the serum (40 $\pm$ 11, n=9) or saline (73 $\pm$ 13, n=6) controls (ANOVA $P<0.05$ and 0.01, respectively) while the WDS induced by 5-MeODMT remained unchanged. Although, the number of 5-MeODMT-induced BMC appeared to be reduced this did not reach significance. Furthermore, the levels of TRH (1.30 $\pm$ 0.17 ng/mg protein, n=9) and CGRP (20.70 $\pm$ 2.96, n=9) after control serum infusion were reduced significantly ($P<0.05$) in the dorsal horn of the cord following TRH-Ab infusion (0.60 $\pm$ 0.17 and 13.22 $\pm$ 1.31, n=6, respectively) but not in the ventral horn. Similarly there were no significant changes in 5-HT, 5-HIAA or ChAT levels in either cord region, although brain stem 5-HIAA levels (4.67 $\pm$ 0.42 ng/mg protein, n=9) were significantly reduced (2.91 $\pm$ 0.25, n=5, $P<0.05$)

The results indicate that i.t. infusion of TRH-Ab alters dorsal horn neuropeptides and TRH- but not 5-HT-evoked WDS, suggesting that separate peptide and amine pathways mediate these behaviours. The absence of changes in the spinal motor behaviour (BMC) and ventral horn markers suggests the possibility that TRH-Ab did not penetrate to the ventral horn in this study.

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EFFECTS OF ALTERED THYROID STATUS ON β-ADRENOCEPTOR BINDING AND ACTION IN SKELETAL MUSCLE

S.J. Richards, G.D. Dimitriadis+, B. Leighton+ and R.A.J. Challiss, Departments of Pharmacology, University of Leicester, Leicester, LE1 9HN, and +Biochemistry, University of Oxford, Oxford, OX1 3QU.

Thyroid hormones influence the metabolic effects of catecholamines in a number of tissues including skeletal muscle (Chu et al. 1985); this 'permissive' effect may be exerted at the level of the receptor, the signal transduction process and/or the metabolic level (Stiles et al. 1984). In the present study, the effects of altered thyroid status on β-adrenoceptor binding, adenylate cyclase activity and inhibition of glycogen synthesis have been determined in skeletal muscle preparations.

Experimental hypothyroidism was induced in rats by administration of 6-n-propyl-2-thiouracil (0.5 mg/ml) in the drinking water for 4-5 weeks. Hyperthyroidism was induced by T_3 administration (0.65 mg/Kg, i.p.) for 10 days. β-adrenoceptor density and affinity was determined using the high affinity antagonist [^{125}I]pindolol (Cook et al. 1987), and adenylate cyclase activity (Chu et al. 1985) were determined in sarcolemmal membranes prepared from red gastrocnemius muscle. Inhibition of glycogen synthesis by the β-adrenoceptor agonist isoprenaline was determined in incubated soleus muscle strips by measuring the incorporation of [U-^{14}C]glucose into glycogen (Challiss et al. 1986).

Saturation analysis of [^{125}I]pindolol binding to sarcolemmal membranes isolated from gastrocnemius muscle of euthyroid (HE) rats revealed a single class of specific binding sites (B_{max} = 14.1 ± 0.7 fmol/mg prot., K_D = 0.13 ± 0.02 nM), hypothyroidism (HO) caused a significant increase in β-adrenoceptor density (B_{max} = 17.7 ± 1.2 fmol/mg prot., $P < 0.05$), whereas hyperthyroidism (HR) caused an insignificant decrease (B_{max} = 11.3 ± 1.5 fmol/mg prot). Altered thyroid status had no effect on antagonist binding affinity. Basal adenylate cyclase activity in skeletal muscle sarcolemmal membranes was unaffected by thyroid status (HE: 37 ± 5 pmol/min/mg prot); however, adenylate cyclase activity in the presence of isoprenaline (10 μM) and GTP (100 μM) was decreased in hypothyroid compared to eu- or hyperthyroid animals (HO: 48 ± 8 vs HE: 70 ± 7, HR: 72 ± 6 pmol/min/mg prot., $P < 0.05$). Furthermore, in the hypothyroid state, the sensitivity of adenylate cyclase activity to stimulation by isoprenaline was also decreased, whereas the concentration-response relationship of adenylate cyclase activity to GTP (in the presence of 10 μM isoprenaline) was unaffected by altered thyroid status. In soleus muscle strips isolated from euthyroid or hyperthyroid animals, isoprenaline (1 μM) caused a maximal 73 ± 3% inhibition of glycogen synthetic rate, whereas isoprenaline caused only a 41 ± 4% inhibition in muscle strips from hypothyroid animals ($P < 0.01$). The decreased responsiveness of this process was accompanied by a 5-fold decrease in the sensitivity to isoprenaline (EC_{50} HE: 1.5 ± 0.5 nM; HO: 7.8 ± 1.2 nM, $P < 0.01$).

We conclude that the effects of altered thyroid status on β-adrenoceptor action in skeletal muscle at the metabolic level (Chu et al. 1985) appear to correlate with changes in adenylate cyclase activity, but not with changes in β-adrenoceptor density, suggesting independent modulation of these opposite effects by thyroid hormones.

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SODIUM LACTATE EFFECTS ON CSF AND SERUM ELECTROLYTES IN RABBITS

P.Glue*, D.T.George[1], R.P.Waxman[1], D.J.Nutt. Reckitt and Colman Psychopharmacology Unit, The Medical School, University Walk, Bristol BS8 1TD, and [1]LCS, NIAAA, Bethesda, MD 20892, USA.

Infusion of sodium lactate provokes anxiety attacks in the majority of patients with panic disorder, but not in normal subjects. This difference does not seem to be due to variations in the effect of lactate on peripheral electrolyte concentrations (Liebowitz et al 1985), so we have studied its action on CSF electrolytes.

Concious rabbits recieved an i.v. infusion of 0.5M sodium lactate in normal saline (N/S) or 5% dextrose in water (D5W) at a dose of 10ml/kg for 20 mins. Blood and CSF samples were taken before and immediately after the lactate infusion, to allow comparison of electrolyte changes. CSF sampling was performed using a chronic indwelling cisternal catheter (Glue et al 1989). Total sodium and chloride were measured by an ion-specific electrode method, and total calcium and magnesium by spectrophotometry. Serum and CSF electrolyte concentrations before and after lactate infusion are shown in the Table.

Lactate in N/S	CSF		Serum	
Electrolyte	Pre-lactate	Post-lactate	Pre-lactate	Post-lactate
Sodium (mmol/l)	155 ± 11	171 ± 27	144 ± 3	159 ± 8***
Chloride "	155 ± 22	168 ± 38	108 ± 3	107 ± 5
Magnesium (mg/dl)	2.3 ± 0.4	2.2 ± 0.4	2.2 ± 0.4	1.9 ± 0.3
Calcium "	4.2 ± 0.5	4.5 ± 0.5***	13.6 ± 0.7	12.8 ± 1.3

Lactate in D5W	CSF		Serum	
Electrolyte	Pre-lactate	Post-lactate	Pre-lactate	Post-lactate
Sodium (mmol/l)	152 ± 4	150 ± 7	146 ± 4	155 ± 9*
Chloride "	138 ± 4	135 ± 3	107 ± 5	99 ± 7***
Magnesium (mg/dl)	2.4 ± 0.3	2.4 ± 0.3	2.2 ± 0.2	1.8 ± 0.2***
Calcium "	4.0 ± 0.3	4.3 ± 0.5**	13.7 ± 0.5	12.2 ± 0.3**

(all values mean ± s.d.; * $p < 0.05$; ** $p < 0.02$; *** $p < 0.01$)

Following infusion of lactate in either N/S or D5W , significant rises in CSF calcium were seen; this may be due to a fall in CSF pH (Dager et al 1988). The increase in sodium in serum is probably due to the increased sodium load from the infusion, and the reduction in serum magnesium, calcium and chloride to a dilution effect. The reduction in serum chloride after D5W infusion is in agreement with recent findings from lactate infusions in humans (George et al 1986).

The disparities between serum and CSF electrolyte responses are unexpected, especially that of a significant increase in CSF calcium, despite a fall in serum calcium. Further studies in humans may be indicated, to see if there are similar CSF/serum disparities, and to see if differences exist between panic patients and normal controls.

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THE EFFECT OF THE TERNARY COMPLEX MECHANISM ON THE PHARMACOLOGICAL ESTIMATION OF AGONIST AFFINITY AND EFFICACY

P. Leff and D. Harper, Department of Pharmacology, Fisons plc - Pharmaceutical Division, Bakewell Road, Loughborough, Leicestershire, LE11 ORH, UK.

According to pharmacological receptor theory, agonist action can be characterised by two quantities, affinity and efficacy (Stephenson, 1956). In practice, these two quantities can be estimated for full agonists using the method of receptor inactivation (Furchgott, 1966) and for partial agonists by comparison with a full agonist (Barlow et al., 1967).

It is now well established that many receptors couple to G-proteins in order to subserve their pharmacological effects. In the case of β-adrenoceptors (De Lean et al., 1978), dopamine (Wregget and De Lean, 1984) and muscarinic (Freedman et al., 1988) receptors it has been proposed that a ternary complex mechanism applies in which the activated state of the receptor is an agonist-receptor-G-protein complex. Recently, it has been shown (Mackay, 1988) that the operation of a ternary complex mechanism may lead to errors in agonist analysis by pharmacological methods. Specifically, when the concentration of G-proteins (denoted here as $[G_o]$) is in excess of the concentration of receptors (denoted here as $[R_o]$), the receptor inactivation method is predicted to produce overestimates of agonist affinity (Mackay, 1988).

This communication reports the results of a more general, theoretical analysis of the effect of the ternary complex mechanism on agonist quantification. It considers a range of conditions relating $[R_o]$ and $[G_o]$, and the effects on partial as well as full agonist analysis. The following predictions are made: (i) reliable estimates of affinity and efficacy can be made using the comparative method under the conditions $[R_o]>>[G_o]$ and $[R_o]<<[G_o]$ whereas the inactivation method only works under the former condition; (ii) errors occur in the estimation of affinity and efficacy by both methods when $[R_o]=[G_o]$ although better estimates are produced by the comparative method; (iii) when errors occur in the absolute estimation of affinity and efficacy, the orders of affinity and efficacy determined by the comparative method will generally be correct; this is unlike the situation for the inactivation method where a high propensity for reversals in affinity order exists; (iv) in general, when the ternary complex mechanism operates the comparative method for partial agonist analysis produces more reliable information for the purposes of receptor classification than does the receptor inactivation method.

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EFFECTS OF LEAD ON THE RAT ISOLATED VAS DEFERENS

H.A. Al-Haboubi*, S. Al-Kasam and K. Elzaroug[1], *Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, U.K. and [1]Department of Pharmacology, Faculty of Pharmacy, University Al-Fateh, Tripoli, Libya.

Conflicting results have been reported on the effects of lead on catecholaminergic function (Shih & Hanin, 1978; Winder, 1982). In the present study, the effects of exposing the vas, for 1 h, to Pb^{2+} 1,4,50 and 100 μM were examined.

Whole vasa were removed from Wistar rats (250-350 g) and suspended in either normal or nominally Ca^{2+}-free Krebs solution at 37°C and isometric contraction recorded. Parallel silver wire electrodes were used for field stimulation. A separate vas was used for each concentration of lead.

The responses to field stimulation (1 to 60 Hz, 0.5 ms, supramaximal volts, for 10 sec) were augmented ($P < 0.05$) by all concentrations of lead used.

4-Aminopyridine (1.1×10^{-4}M) alone produced 56% while Pb^{2+} 50 μM produced 30% increase in areas under frequency-response curves calculated by a microcomputer implementation (Al-Haboubi & Zeitlin, 1983). The combination of these agents produced 252% ($P < 0.05$) increase in this response. Matsumoto & Riker (1984) suggested that 4-aminopyridine enhances neurotransmitter release from vas deferens by facilitating Ca^{2+} entry into the synaptic terminals. However, the effect of tyramine (3.7×10^{-7} M) was also significantly enhanced by all concentrations of lead used in this study.

The contraction produced by exogenous noradrenaline (NA) (1.2×10^{-8}M) was not significantly affected by Pb^{2+} 1 and 50 μM, reduced (40%, $P < 0.05$) by 4 μM and was increased (124%, $P < 0.05$) by 100 μM. In normal Krebs solution, the contraction produced by 15 mM KCl was increased by 81 and 135% in presence of Pb^{2+} 1 and 100 μM respectively, but was unaffected by 4 and 50 μM. Measuring the time required for NA and KCl contractions to decline to half maximum, it was found that all concentrations of lead did not affect this time in KCl-induced contraction. In NA-induced contraction, only Pb^{2+} 1 and 4 μM increased this time by 164% ($P < 0.025$) and 90% ($P < 0.01$) respectively.

In nominally Ca^{2+}-free Krebs solution, the contraction produced by cummulative addition of Ca^{2+} (1 to 15 mM) in presence of 80 mM KCl was not significantly affected by lead. In this condition, verapamil (2.2×10^{-9} M) inhibited the Ca^{2+}-induced contraction to the same extent, both in presence and absence of Pb^{2+} 1 and 4 μM .

These results suggest that the main effect of lead in this preparation is to increase transmitter release. In addition to this, lead appears to have also some complex postsynaptic actions.

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LOCALISATION OF [^{3}H]-MK-801 BINDING SITES IN RAT BRAIN FOLLOWING LESIONS OF STRIATUM OR SUBSTANTIA NIGRA

A.Hudson* & G.N. Woodruff, Neuroscience Research Centre, Merck Sharp and Dohme Research Laboratories, Terlings Park, Eastwick Road, Harlow, Essex, CM20 2QR

MK-801 is a potent anticonvulsant and neuroprotective agent. These actions are thought to be due to its ability to antagonise N-methyl-D-aspartate (NMDA) receptors at the level of the associated ion channel (Gill et al, 1987; Kemp et al, 1986). Autoradiography of tritiated MK-801 reveals a heterogenous distribution of binding sites throughout the mammalian CNS (Bowery et al, 1988). We report here on the autoradiography (ARG) of [^{3}H]-MK-801 binding in rats with lesions of intrinsic striatal neurones induced by direct injection of quinolinate into the right striatum, compared to control rats and rats treated to remove the dopaminergic striatal input, by injection of 6-Hydroxydopamine (6-OHD) into the right substantia nigra.

Degeneration of the right nigrostriatal pathway was obtained by unilateral injection of 6-OHD (8 µg) in the substantia nigra pars compacta (Stoessl et al, 1987). Direct injection of quinolinate (200 nmol) in 1µl phosphate buffered saline in to the right striatum was performed according to the method of Schwarcz et al (1983). Following 2 weeks recovery, brains were prepared for MK-801 ARG, 12µm cryostat cut sections were incubated in 50mM Tris-HCl (pH7.4, 20°C,, 20min) containing 10nM [^{3}H]-(+)-MK-801 (NEN) and non-specific binding defined by the addition of 10µM thienylcyclohexyl-piperidine (TCP). After rinsing in fresh buffer sections were rapidly dried before apposition to [^{3}H]-sensitive film with [^{3}H]-brain paste standards for 4 weeks (Bowery et al, 1988). Resulting autoradiograms were analysed on a Quantimet 970 image analysis system (Cambridge Instruments). Specific [^{3}H]-MK-801 binding in left and right caudate putamen are shown in Table 1, left and right values were subject to a paired Student's t-test.

Table 1

Group	Specific Binding fmol/mg wet tissue left caudate putamen	right caudate putamen	p
Sham lesion	27.9 ± 4.13	26.1 ± 4.56	ns
6-OHD	27.54 ± 6.87	29.9 ± 7.47	ns
Sham lesion*	34.8 ± 2.79	33.47 ± 7.42	ns
Quinolinate*	29.92 ± 2.97	9.58 ± 2.04	p<0.001

Mean ±-SEM, triplicate measurements on 3 sections from 3 or 4(*) animals.

In conclusion MK-801 recognition sites are unlikely to be located on dopaminergic terminals in the rat striatum as they are unaffected by a nigral lesion. In contrast, destruction of intrinsic cell bodies by quinolinic acid significantly decreases the density of binding sites in the caudate putamen. Further study will be required to determine the location of the remaining binding sites which may be on terminals of the cortical-striatal pathway.

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THE SULPHONIC AND SULPHINIC AMINO ACID CONTENTS OF IMMATURE SPINAL CORD AND CAUDATE-PUTAMEN OF THE RAT

Sarah J. Waller, I.C. Kilpatrick and R.H. Evans, Department of Pharmacology, School of Medical Sciences, University Walk, Bristol BS8 1TD.

Cysteic acid (CA), cysteine sulphinic acid (CSA), homocysteic acid (HCA) and homocysteine sulphinic acid (HCSA) are potent putative excitatory amino acid transmitters (Mewett et al, 1983). The immature rat spinal cord is a robust, synaptically active in vitro preparation suitable for investigation of a functional role of these and other neurotransmitter compounds in primary afferent nerves. However, there is no data in the literature regarding the levels of these compounds in this tissue. We report now a method which simultaneously quantifies the amounts of these four sulphur-containing excitatory amino acids in rat spinal cord and brain.

Male Wistar rats (neonatal 4-5 days old or adult 45-60 days old) were anaesthetised with urethane 1.8g/kg i.p., and following decapitation, spinal cords (SpC) or caudate-putamen (CP) were rapidly dissected onto dry ice. The CP were also dissected from adult rats which were stunned rather than anaesthetised with urethane. Tissue samples were assayed for amino acid content using a reversed phase HPLC method modified from that of Kilpatrick and Mozley (1986). The sulphur-containing amino acids were identified by comparing tissue samples with samples that had been "spiked" with authentic amino acids, and the results are presented in Table 1.

Table 1. Contents of CSA and HCA in rat spinal cord and caudate-putamen.

	n	CSA	HCA
Neonatal SpC	5	2.53 ± 0.20	1.81 ± 0.22
Adult CP	4	11.90 ± 0.62	2.54 ± 0.29
Adult CP (no anaesthesia)	4	11.47 ± 0.59	2.19 ± 0.09

Values represent the mean ± S.E.M. in pmol/mg wet weight.

Neither CA nor HCSA were detectable in any of the samples (< 0.75 and < 0.1 pmol/mg respectively). Although Ida and Kuriyawa (1983) have reported the presence of CA in rat CP, their method involved homogenisation with trichloroacetic acid and ether extraction, a procedure which in our laboratory has been found to oxidise endogenous CSA to CA (Waller, unpublished). From the lack of effect of urethane anaesthesia on CSA or HCA in CP, we inferred that this anaesthetic was having no effect on the levels in the spinal cord.

These results suggest that of the four excitants only CSA and HCA should be considered as neurotransmitter candidates in rat spinal cord or caudate-putamen, HCA being a potential NMDA agonist and CSA interacting with non-NMDA receptors (Mewett et al, 1983).

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QUANTITATIVE PHARMACOLOGICAL EVALUATION OF THE RECEPTOR SELECTIVITY OF EXCITATORY AMINO ACID AGONISTS

P.J. Birch, A.J. Baxendale and A.G. Hayes, Department of Neuropharmacology, Glaxo Group Research, Ware, Herts, SG12 ODP.

Receptors which mediate the depolarising actions of excitatory amino acids (EAAs) can be divided into those sensitive to N-Methyl-D-Aspartate (NMDA) and those sensitive to (RS)-α-amino-3-hydroxy-4-methyl-5-isoxazole-propionate (AMPA) and kainate (non-NMDA). Whilst agonists with apparent selectivity for NMDA and non-NMDA sites have been described (Davies et al., 1982), a detailed quantitative pharmacological evaluation of the receptor selectivity of these compounds is lacking. Thus, the present study was designed to characterise more fully the receptor selectivity of a series of EAA agonists.

Hemisected spinal cords from 2-7 day-old Sprague-Dawley rats were set up for conventional grease-gap recording in a two compartment bath as previously described (Birch et al., 1988). Both compartments were independently superfused with oxygenated Mg^{++}-free Krebs-Ringer of the following composition (mM): NaCl 118, KCl 4.7, $NaHCO_3$ 25, $CaCl_2$ 1.3, KH_2PO_4 1.2, glucose 11.1; pH 7.4, at room temperature. TTX (30nM) was also present. Agonists were applied to one side of the bath and concentration-response curves constructed using a semi-cumulative procedure. The following antagonists were used to define receptor selectivity: FG9065, 10μM, 15-min pre-equilibration (non-NMDA selective, Honoré et al., 1988) and MK801, 10μM, 75-min pre-equilibration (NMDA-selective, Wong et al., 1987).

FG9065 (10μM) produced parallel shifts in the concentration-response curves to AMPA, kainate, quisqualate, willardiine, 5-bromowillardiine and ß-oxalylamino-L-alanine (L-BOAA), (concentration-ratios were: 14±1 (n=6), 19±5 (n=5), 10±2 (n=4), 16±3 (n=4), 15±2 (n=4) and 11±1 (n=4), respectively), but produced little antagonism of the responses to NMDA, ibotenate, L-glutamate, D-glutamate, L-aspartate, D-aspartate and cis-(±)-1-amino-1,3-cyclo-pentanedicarbotylate (cis-ACPD). Schild plots for antagonism by FG9065 of responses to AMPA, kainate, quisqualate, willardiine, 5-bromowillardiine and L-BOAA were constructed; pA_2 values (95% confidence limits) were 6.2 (5.9-6.4), 6.1 (5.5-6.6), 6.0 (5.9-6.1), 6.3 (6.1-6.4), 6.2 (6.1-6.3) and 6.0 (5.8-6.1), respectively (n=3-4). Schild slopes were not significantly different from unity. MK801 (10μM) completely abolished responses to NMDA, ibotenate and cis-ACPD, produced parallel shifts in the concentration-response curves to L-glutamate, D-glutamate, L-aspartate, and D-aspartate (concentration-ratios were 2.1±0.2 (n=4), 13±2 (n=4), 3±1 (n=8) and 6±1 (n=3), respectively) and failed to antagonise the responses to AMPA, kainate, quisqualate, willardiine, 5-bromowillardiine and L-BOAA. Addition of 10μM FG9065, in the presence of MK801, produced further shifts in the concentration-response curves to D-glutamate, L-aspartate and L-glutamate but not to D-aspartate.

The results show that willardiine, 5-bromowillardiine and L-BOAA are non-NMDA selective along with AMPA, kainate and quisqualate, whereas ibotenate and cis-ACPD are NMDA-selective. L-glutamate, D-glutamate, L-aspartate and D-aspartate are relatively non-selective, producing depolarisations via an action at NMDA and non-NMDA receptors and by an as yet uncharacterised mechanism.

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INHIBITION OF CONVULSIONS INDUCED BY CENTRALLY-ADMINISTERED NMDA IN MICE

J.L. Moreau & L. Pieri, Pharmaceutical Research Department, F. Hoffmann-La Roche & Co., Ltd., CH-4002 Basle, Switzerland.

N-methyl-D-aspartate (NMDA) receptors have been found to play a key role in the genesis of epileptiform activity (Schwarcz & Meldrum, 1985), neurodegenerative disorders (Schwarcz et al., 1984) and neurotoxic effects of ischemia/hypoxia (Rothman & Olney, 1987). In the present study, we describe a very simple and efficient method for testing drugs that could antagonize NMDA-induced convulsions.

After peripheral (i.p. or p.o.) pretreatment with drugs to be tested or vehicle, NMDA (1 nmol/1 µl) was injected in the lateral ventricle of conscious mice according to Clark et al. (1968). For 5 min post-NMDA injection mice were observed for appearance of wild running, clonic and/or tonic convulsions. Following vehicle pretreatment and then NMDA injection, these behavioral items typically appeared in about 90% of the animals with a latency of 5 to 20 s.

Tonic, and to a lesser extent clonic, convulsions were inhibited by such NMDA antagonists as MK 801 (0.1-0.3 mg/kg), phencyclidine (PCP: 0.1-3 mg/kg), thienylcyclohexylpiperidine (TCP: 0.1-3 mg/kg), carboxypiperazine-propyl-phosphonic acid (CPP: 1-10 mg/kg), but also by a variety of anticonvulsants and/or minor tranquilizers such as phenobarbital (5-30 mg/kg), diphenylhydantoin (1-30 mg/kg), meprobamate (100-200 mg/kg) and sodium valproate (100-300 mg/kg). Dextromethorphan (10-30 mg/kg) and dextrorphan (3-30 mg/kg) were also active. Among the classical benzodiazepine receptor (BZR) agonists, triazolam (0.001-0.05 mg/kg) and clonazepam (0.01-0.3 mg/kg) were the most effective; diazepam (0.05-3 mg/kg) and Ro 16-6028 (a BZR partial agonist, 0.05-10 mg/kg) were of the same order of potency as MK 801. Flumazenil (a BZR antagonist, 1-30 mg/kg), muscimol (0.1-0.3 mg/kg) and THIP (1-3 mg/kg) (both GABA agonists) were without effect. Baclofen (1-5 mg/kg) was slightly active in preventing tonic convulsions but not clonic convulsions. Flumazenil (10 mg/kg) antagonized the protective effect of diazepam (3 mg/kg), but not of MK 801 (1 mg/kg).

In conclusion, convulsions induced by i.c.v. NMDA offer a very simple and very reproducible method to detect anticonvulsant drugs. The i.c.v. route allows the injection of a low amount of NMDA as compared to the huge dose required to induce convulsions when NMDA is given i.p. (Ferkany et al., 1988). In spite of the lack of selectivity for NMDA antagonists, this method could be fruitfully used for the early detection of new anticonvulsants and/or compounds active in ischemic/hypoxic brain insults.

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COMPARISON OF THE PHARMACOLOGICAL PROFILES OF FOUR NMDA ANTAGONISTS, IFENPRODIL, SL 82.0715, MK-801 AND CPP, IN MICE

G. Perrault, E. Morel, D.J. Sanger & B. Zivkovic. Introduced by K.G. Lloyd.
SYNTHELABO RECHERCHE (L.E.R.S.), 31 Ave P.V. Couturier, 92220 Bagneux, France.

The pharmacological profiles of the cerebral anti-ischaemic agents, ifenprodil and its derivative SL 82.0715 [(±)-α-4-chlorophenyl(4-fluoro phenylmethyl)-4 piperidine-1-ethanol)hydrochloride], which possess NMDA antagonist properties (Gotti et al, 1988; Carter et al, 1988), were compared with those of MK-801 and CPP. These latter compounds are potent NMDA antagonists, CPP behaving as a competitive antagonist and MK-801 a non-competitive antagonist blocking the NMDA receptor-associated ion channel.

Anticonvulsant effects were evaluated by observing the presence of tonic convulsions provoked by maximal electroshock (60mA, 50Hz, 0.4s) and clonic convulsions induced by intracerebroventricular injection of NMDLA (10nmoles/ mouse). Central depressant effects were assessed by measuring spontaneous locomotor activity recorded in photocell activity cages, and motor performance in the rotarod and loaded grid tests.

	ED_{50}, mg/kg, i.p.			
	Ifenprodil	SL 82.0715	CPP	MK-801
Electroshock convulsions	29	15	2	0.1
NMDLA convulsions	23	26	4	0.2
Locomotor activity	7 (decrease)	10 (decrease)	34 (decrease)	0.1 (increase)
Rotarod	29	59	10	(stimulation)
Loaded grid	34	≥100	5	(stimulation)

The four compounds possess anticonvulsant properties as shown in the table. The order of potency is MK-801 > CPP >> SL 82.0715 and ifenprodil. Only CPP showed muscle relaxant effects at doses very close to those producing anti-convulsant activity. Ifenprodil and SL 82.0715 did not affect performance in the loaded grid test at doses 2 or 3 times greater than those active against convulsions. However, ifenprodil and SL 82.0715 decreased locomotor activity at doses lower than anticonvulsant doses; CPP also decreased this behaviour but at doses higher than those that were anticonvulsant. In contrast, MK-801 increased locomotor activity at doses very similar to those inducing anticonvulsant effects. It was not possible to obtain meaningful values for the effect of this compound in the rotarod and loaded grid tests because its marked stimulant properties caused mice to jump from the apparatus. Although ifenprodil and its derivative SL 82.0715 have NMDA receptor antagonist properties, they do not share all the activities induced in mice either by MK-801 or by CPP. The differences observed quantitatively in their anticonvulsant effects and qualitatively in their effects on locomotor activity and motor performance indicate that a distinct mechanism could be involved in the action of these compounds as has been suggested for their biochemical profiles (Carter et al, 1988) and for their behavioural effects (Jackson and Sanger, 1988; Sanger and Zivkovic, 1988).

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CGP 37849/CGP 39551: COMPETITIVE NMDA RECEPTOR ANTAGONISTS WITH POTENT ORAL ANTICONVULSANT ACTIVITY

M. Schmutz[1], K. Klebs[1], H.-R. Olpe[1], H. Allgeier[1], R. Heckendorn[1], C. Angst[2], D. Brundish[2], J.G. Dingwall[2] & G.E. Fagg[1] (introduced by L. Maitre), [1]Pharmaceutical Research Department and [2]Central Research Laboratories, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland.

Evidence from many investigations indicates that N-methyl-D-aspartate (NMDA) receptor antagonists may be effective agents for the treatment of epilepsy. NMDA receptor mechanisms have been shown to be involved in burst firing and the generation of seizure activity, and competitive and non-competitive antagonists can prevent convulsions in animal models of epilepsy. However, the therapeutic use of non-competitive NMDA receptor antagonists seems likely to be limited by their side-effect profiles, and orally-active competitive antagonists have been unavailable to date. Here we describe the anticonvulsant profiles of a novel competitive NMDA receptor antagonist, CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; 4-methyl-APPA), and its carboxyethylester, CGP 39551.

Seizures were induced by electroshock (rats, 50 Hz, 36 mA, 0.63 s; mice, 50 Hz, 16 mA, 0.2 s), i.p. administration of pentylenetetrazol (mice, 70 mg/kg), or by electrical kindling of the amygdala via chronically-implanted electrodes in rats. Electrophysiological studies *in vivo* were conducted in rats anaesthetized with chloral hydrate.

Table 1. Antagonism of electroshock-induced seizures in rats by CGP 37849 and CGP 39551.

Time after drug treatment (h)	CGP 37849		CGP 39551	
	p.o.	i.v.	p.o.	i.v.
0.5	NT	1	NT	>12
1	>60	1	>20	5
4	8-22	3	8	4
8	25-28	>8	8	7
24	100	NT	21	NT

Values are ED_{50}'s (mg/kg, determined by the method of Spearman-Kaerber) for protection against tonic convulsions of the hind limbs. NT, not tested.

Both CGP 37849 and CGP 39551 were potent anticonvulsants when evaluated against electroshock-induced seizures in rats and mice. However, optimal activity was observed to depend markedly on the route and time of drug administration. For CGP 37849, minimal ED_{50} values following oral administration were 8-22 mg/kg in rats (Table 1) and 13-21 mg/kg in mice; CGP 39551 was slightly more potent, with values of 8 mg/kg (rats) and 4 mg/kg (mice). After i.v. or i.p. treatments in both species, minimal ED_{50}'s of 0.4-1.9 mg/kg were determined for CGP 37849, and 3-6 mg/kg for CGP 39551. Following oral administration, the duration of action of CGP 37849 was about 8 h, while CGP 39551 still showed significant activity after 24 h (ED_{50}'s 9-21 mg/kg; Table 1). Both compounds were anticonvulsant at doses below those at which overt side-effects were apparent. CGP 39551 (but not CGP 37849) additionally delayed the development of kindled seizures in rats at doses of 10 mg/kg p.o. and above, and exhibited weak anticonvulsant activity against pentylenetetrazol-evoked seizures. Anticonvulsant doses of both drugs selectively antagonized iontophoretically-applied NMDA-, but not quisqualate- or kainate-evoked neuronal firing in the rat hippocampus *in vivo*.

In conclusion, CGP 37849 and CGP 39551 are the first competitive NMDA receptor antagonists to show significant oral anticonvulsant activity and, as such, are promising candidates for novel antiepileptic therapy in man.

CGP 37849: CHARACTERIZATION OF A NOVEL AND POTENT COMPETITIVE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR ANTAGONIST

G.E. Fagg[1,3], M.F. Pozza[3], H.-R. Olpe[3], F. Brugger[3], P. Baumann[3], H. Bittiger[3], M. Schmutz[3], C. Angst[2], D. Brundish[2], H. Allgeier[3], R. Heckendorn[3] & J.G. Dingwall[2] (introduced by L. Maitre), [1]Friedrich Miescher Institute, [2]Central Research Laboratories and [3]Pharmaceutical Research Department, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland.

NMDA receptor mechanisms have been shown to participate in a number of pathological phenomena in the vertebrate central nervous system, and antagonists with appropriate potency, selectivity and bioavailability have been predicted to fulfil a variety of therapeutic roles. Here, we describe a novel analogue of the known antagonist, 2-amino-5-phosphonopentanoate (AP5), that is a potent competitive NMDA receptor blocker and is centrally-active following oral administration.

Radioligand binding assays using rat brain membranes, investigations of L-glutamate uptake using crude synaptosomal preparations, and electrophysiological experiments using rat hippocampal slices were all conducted according to established procedures. Electrophysiological studies *in vivo* were performed using rats anaesthetized with chloral hydrate.

Table 1. Inhibition of L-glutamate binding and CPP binding by AP5 analogues.

Substance	K_i value (uM)	
	L-^{3}H-glutamate binding	^{3}H-CPP binding
CGP 37849	0.22 ± 0.04	0.035 ± 0.020
CGS 19755	0.84 ± 0.39	0.13 ± 0.02
CPP	0.82 ± 0.05	0.17 ± 0.02
DL-AP5	1.5 ± 0.2	NT
D-AP5	0.88 ± 0.45	0.26 ± 0.05
L-AP5	29 ± 9	4.9 ± 0.7
NMDA	5.3 ± 0.9	7.7 ± 0.4
L-glutamate	0.20 ± 0.03	0.64 ± 0.05

K_i values are means ± S.E.M. of data from 3-5 independent experiments. CPP, 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonate; NT, not tested.

Table 1 shows that, in NMDA receptor binding assays, CGP 37849 (DL-(E)-2-amino-4-methyl-5-phosphono-3-pentenoic acid; 4-methyl-APPA) was the most potent inhibitor amongst a series of compounds known to be active at the NMDA receptor, with a Ki of only 35 nM at CPP binding sites. It was weak or inactive in assays for 17 other receptors, including the quisqualate and kainate types of excitatory amino acid receptor, and at concentrations up to 1 mM had no effect on the synaptosomal uptake of L-glutamate. In the hippocampal slice preparation, concentrations of CGP 37849 in the range 0.3-3 uM antagonized NMDA- but not quisqualate- or kainate-evoked increases in pyramidal cell firing, suppressed the multiple population spikes evoked in CA1 neurons by Schaffer collateral stimulation (low Mg^{2+}-containing medium), and prevented the induction of long-term potentiation in area CA1. In anaesthetized rats, oral administration of CGP 37849 selectively blocked the increases in hippocampal neuronal firing evoked by iontophoretically-applied NMDA, but not by quisqualate or kainate.

CGP 37849 thus is a potent, orally-active NMDA receptor antagonist with which to examine the roles of NMDA receptor-mediated events in brain function, and is a potential candidate for the treatment of disorders such as epilepsy and ischaemic neurodegeneration.

RILUZOLE ANTAGONISES EXCITATORY AMINO ACID-EVOKED FIRING IN RAT FACIAL MOTONEURONES IN VIVO

D. Girdlestone, A. Dupuy, A. Coston+, L. Roy-Contancin++ and D. Escande. (Introduced by A. Doble). Laboratory of Cellular Electrophysiology. Rhône-Poulenc Santé, CRV, 13 quai Jules Guesde, BP 14, 94403 Vitry sur Seine. France.

Biochemical and behavioural models in which riluzole, (2-amino-6-trifluro-methoxybenzothiazole, PK 26124, RP 54274) displays glutamate antagonist activity have been reported (Mizoule et al; 1985, Benavides et al; 1985) but the mechanism of action is unknown. To investigate the effects of the drug on single neurones, normally quiescent motoneurones of the rat facial nucleus whose pharmacology is known (McCall et al; 1979; 1980; Roy-Contancin, 1987) were chosen.

Male Sprague-Dawley rats weighing 250-350 g were anaesthetised with urethane (1.25 g/kg) and either an i.p. or i.v. (jugular) cannula inserted. Seven barrelled microelectrodes were used for extracellular single unit recording and microiontophoresis. The barrels were filled with combinations of the following solutions: 3M NaCl (recording) 4M NaCl (current balancing), NMDA (50mM in 165 mM NaCl, pH 8), kainate (KA, 5mM in 165 mM NaCl, pH 8), quisqualate (QI, 5mM in 165 mM NaCl, pH 8), sodium-L-glutamate (0.2 M, pH 8), L-aspartate (0.1 M, pH 8), PSB (saturated solution).

I.p. or i.v. admnistration of riluzole depressed firing in facial motoneurones evoked by microiontophoretic application of NMDA, KA and QI. All three agonist responses were equally depressed. The effect of i.p. administration of doses of 0.3 and 0.6 mg/kg of riluzole became significant 20 minutes after injection and reached a maximum 30 minutes post-injection. A 100% depression of response amplitude was observed with the higher dose of 0.6 mg/kg (n=6). Full recovery from the effects of either dose was not observed in these experiments. In one study, the effects of riluzole directly on the endogenous amino acids (EAAs), glutamate and aspartate, were observed. With 1.0 mg/kg i.p., there was a 100% depression of the activity evoked by both transmitters. At doses of 0.3 (n=5) and 0.6 (n=3) mg/kg i.v. similar magnitudes of depression of evoked activity were seen to those observed with i.p. injection. The onset of action was between 7 and 9 minutes and in 3 out of 5 animals injected with 0.3 mg/kg there was a partial recovery which was apparent between 30 and 50 minutes post-injection.

Riluzole has a relatively rapid onset of action, and a potent and reversible depressant action on neuronal responses to EAAs, probably post synaptically. It should now be valuable to investigate the regional and transmitter system specificity or sensitivity of this action of riluzole.

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ELECTROPHYSIOLOGICAL STUDIES OF THE EFFECTS OF RILUZOLE ON PURKINJE CELLS IN CEREBELLAR SLICES

M. W. Debono and D. Escande. (Introduced by A. Doble). Rhône-Poulenc Santé, CRV, BP 14, 13 quai Jules Guesde, 94403 Vitry sur Seine. France.

Riluzole (2-amino-6-trifluoromethoxy benzothiazole, PK 26124, RP 54274) is a new compound which has been shown to possess glutamate antagonist properties in biochemical models both *in vivo* and *in vitro* in the rat cerebellum (Benavides et al., 1985). This compound has also been demonstrated to inhibit transmitter release from cultured cerebellar granule cells (Drejer et al; 1986). Since excitatory amino-acids (EAAs) are known to be involved in fast synaptic transmission in the cerebellum (Chujo et al., 1975; Crepel et al., 1982), we have investigated the effects of riluzole on Purkinje cells (PCs) and their synapses in cerebellar slices maintained *in vitro*.

Either immature (7-13 days old) or adult (45 days old) rats were used in all experiments. Sagittal cerebellar slices (400 µm) from the vermis region were placed in a recording chamber superfused with a prewarmed Krebs-Ringer solution under an atmosphere of 95% O_2-5% CO_2. Glass recording electrodes were filled with either 3M KCl (40-80 MΩ) for intracellular studies or 4M NaCl (5-10 MΩ) for extracellular recordings. The responses of PCs to L-glutamate (100 µM) and its modification by riluzole were studied with short duration (1-1.5 minutes) applications of the EAA. High concentrations of riluzole (1-100 µM) were applied with short periods whereas lower concentrations (10 nM-10 µM) were applied with longer periods of superfusion (application time: 5-40 minutes).

Riluzole exerted a dose-dependent (5-100 µM) inhibition of the extracellularly recorded spontaneous activity of immature PCs (n=43), reaching a maximum of 70% ± 5% inhibition at a concentration of 100 µM. Similar observations were made in adult rats (n=6). At a concentration of 5 µM, which only slightly affected spontaneous activity, riluzole partially blocked the response of PCs to superfused L-glutamic acid (100 µM).

In adult rats, synaptic and antidromic activation of PCs could be obtained by stimulating the white matter at the base of the folia (climbing fibres), or the upper part of the molecular layer (parallel fibres). The effect of riluzole on excitatory post-synaptic potentials (epsps) generated by stimulation of these two pathways, whose transmitters are thought to be EAAs, was investigated. For climbing afferents, the threshold concentration was 0.1 µM and complete inhibition was seen at 10 µM. The effects of low concentrations were reversible (i.e. 10 minutes after start of wash-out, recovery was apparent). With higher concentrations (≥10 µM) recovery was rarely seen (n=19). Preliminary data suggests that parallel fibre responses (n=3) are similarly depressed.

These findings that riluzole suppressed the glutamate mediated response of PCs to synaptic activation provide a further example of the activity of this compound at glutamatergic synapses.

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REGULATION OF N-METHYL-D-ASPARTIC ACID-STIMULATED [^{3}H]-ACETYLCHOLINE RELEASE FROM RAT STRIATAL SLICES BY PRESYNAPTIC MUSCARINIC RECEPTORS

Michel Didier, M. Heaulme, N. Gonalons, J.P. Chambon, P. Soubrié. Neurobiology Department, Sanofi Recherche, Rue du Pr Blayac, Montpellier, France.

Excitatory amino acids elicit the release of (3H)acetylcholine (ACh) from rat striatal slices by acting at NMDA preferring receptors (Lehmann and Scatton, 1982). Since, in various part of the CNS, (3H)ACh release is known to be subjected to a classical inhibitory mechanism involving M2 cholinergic receptors [Raiteri et al., (1984), James and Cubeddu (1987)] the presence of such a modulation on the stimulatory action of NMDA on striatal cholinergic neurons was examined and compared to that produced by K+-induced depolarization.

NMDA (0.1 mM) and K+ (35 mM) stimulated (3H)ACh release was performed as described by Lehmann and Scatton (1982) using striatal slices incubated in oxygenated normal Krebs buffer containing 50 nM N-methyl-3H-choline for 30 min. Slices were then continuously perfused at 0.5 ml/min with Krebs medium in which MgCl2 was omitted and containing hemicholinium (10 µM). After 42 min of perfusion, 3 min perfusate samples were collected and their radioactive content estimated. Tissue was exposed to the drugs 9 min before and during a 3 min stimulation with NMDA or K+.

Carbachol and oxotremorine, two cholinergic agonists, concentration-dependently reduced (3H)ACh release induced by NMDA and K+ with comparable efficacy (Table 1). In each case, maximal inhibition (100 %) was reached at 0.1 mM for both cholinergic agonists.

Table 1 : Inhibitory effect of cholinergic agonists on the release of (3H)ACh stimulated by 0.1 mM NMDA or 35 mM K+

	Inhibition of (3H)ACh release IC50 (µM) - (Mean ± s.e.mean)	
Compounds	Induced by NMDA	Induced by K+
Carbachol	3.0 ± 0.37	4.0 ± 0.42
Oxotremorine	1.2 ± 0.17	1.0 ± 0.32

In both kinds of (3H)ACh stimulation (NMDA or K+) the inhibitory effect observed in the presence of carbachol (0.01 mM) was reversed by cholinergic antagonists with similar IC50 values : pirenzepine (0.8 vs 0.7 µM), atropine (6 vs 9 nM). Conversely, the inhibition of NMDA-stimulated (3H)ACh release by competitive antagonists such as (±)AP7, d(-)AP5 and (±)CPP, IC50 values being 100 µM, 50 µM and 1.0 µM, respectively, was not affected by 0.01 mM atropine or pirenzepine. Moreover, neither D(-)AP5 nor (±)CPP at 0.1 mM reduced K+ evoked (3H)ACh release. NMDA did not affect specific (3H)QNB binding nor did cholinergic agonists alter specific (3H)CPP binding to whole brain membranes. Finally, tetrodotoxin concentration-dependently inhibited NMDA-stimulated (3H)ACh release (IC50 = 6 nM) but decreased the K+-evoked (3H)ACh release only by 30 % at 1 µM without affecting the modulatory effect of the muscarinic agonists or antagonists.

In conclusion, these results suggest the presence of muscarinic presynaptic receptors which modulate NMDA and K+ stimulated (3H)ACh release in rat striatum but exclude the possibility of a direct or indirect interaction between NMDA preferring binding sites and cholinergic receptors.

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NMDA RECEPTORS AND LEARNING: PARALLEL DOSE-RESPONSE IMPAIRMENT OF SPATIAL LEARNING AND LTP IN VIVO BY D-AP5

S. Davis, S.P. Butcher & R.G.M. Morris, Department of Pharmacology, University of Edinburgh Medical School, George Square, Edinburgh, EH8 9JZ.

Several lines of research suggest the involvement of hippocampal N-methyl-D-aspartate (NMDA) receptors in certain types of learning. These are: (1) In hippocampus, 2-amino-5-phosphonopentanoate (AP5) blocks long term potentiation (LTP: Collingridge et al, 1983), whose cellular mechanisms are potential substrates for learning, (2) Chronic intraventricular infusion of 40mM D,L-AP5 impairs the performance of rats in spatial learning tasks, but does not impair visual discrimination (Morris et al, 1986). A similar behavioural profile is seen in hippocampectomised rats (Morris et al, 1982). Supportive evidence indicates that the hippocampus has the highest density of NMDA receptors in the rat forebrain (Monaghan & Cotman, 1986). The purpose of this study was to examine, in the same animal, the dose-response profile of D-AP5 (5mM, 13mM, 30mM) on (1) spatial learning, (2) induction of LTP, (3) brain tissue concentration of AP5.

Male Lister hooded rats (N = 19) were surgically implanted, under tribromoethanol anaesthesia ($0.29gm/kg^{-1}$), with osmotic minipumps containing one of the concentrations of AP5 or artificial CSF. The pumps continuously infused drug or aCSF into the right ventricle over 14 days (0.5µl/hr). During this time, the animals were (1) tested for their ability to use spatial cues in learning the location of a fixed but hidden escape platform in an open-field water-maze, and then (2) tested for LTP in vivo by recording perforant path-evoked potentials in the dentate hilus in urethane anaesthetised rats (1.5 gm/kg). Low frequency stimulation (0.1Hz, 100µS, 7V) was interspersed with a high frequency tetanus (400 Hz, 3 x 33 impulses, 7V). Finally, (3) tissue taken from the right and left hippocampus, right and left visual cortex and frontal cortex was analysed for AP5 content using HPLC.

Analysis of variance of latencies taken to locate the platform showed a dose-related impairment ($p < 0.05$). The performance of animals treated with 5mM D-AP5 was similar to controls whereas an impairment was seen in animals infused with 30mM D-AP5. The impairment was a slowing down of spatial learning rather than the blockade seen with hippocampal lesions. The amount of LTP that could be triggered showed a similar dose response function to that seen behaviourally ($P < 0.05$). At 5mM the amount of LTP evoked was the same as that seen in controls, whereas at 30mM no LTP could be triggered. Variable performance in both behavioural and electrophysiological tests was noted in animals treated with 13mM D-AP5. Preliminary analysis of the tissue content of AP5 in hippocampi from these animals indicates that the mean concentrations were: 5mM = 0.063 ± 0.026nmol/mg wet wt., 13mM = 0.134 ± 0.03nmol/mg wet wt., 30mM = 0.544 ± 0.178nmol/mg wet wt.

The results indicate that the dose-response function for blocking LTP in vivo is similar to that for its behavioural effects upon spatial learning. As no dose of AP5 was found to block LTP but have no behavioural effects, the hypothesis that hippocampal NMDA receptors may be activated during spatial learning remains tenable.

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6,7-DINITROQUINOXALINE-2,3-DIONE (DNQX) BLOCKS MONOSYNAPTIC EXCITATIONS IN THE MOUSE OLFACTORY CORTEX SLICE

G.G.S. Collins & K.S. Buckley, University Department of Pharmacology and Therapeutics, Royal Hallamshire Hospital, Sheffield S10 2JF

The major peripheral input into the mammalian olfactory cortex is provided by the fibres of the lateral olfactory tract (LOT) which are thought to utilise either aspartate or glutamate as their transmitter (Collins, 1986). Non-selective antagonists of excitatory amino acid receptors effectively block transmission at the LOT-pyramidal cell synapse whereas antagonists selective for the N-methyl-D-aspartate (NMDA) receptor category are inactive : this led to the proposal that receptors of the quisqualate (Q) or kainate (K) categories might mediate monosynaptic excitations in the olfactory cortex (Collins, 1982). The recent synthesis of the quinoxalinediones, which selectively antagonize Q and K receptors (Honore et al., 1988), allows this hypothesis to be tested.

Mouse olfactory cortex slices (400μm thick) were preincubated and perfused at room temperature (Pickles & Simmonds, 1976). In the first series of experiments, submaximal depolarizations evoked by L-aspartate (5mM), Q (either 0.0625 or 0.125mM), K (0.05mM) and NMDA (0.05mM) were measured using extracellular electrodes (Brown & Galvan, 1979). DNQX (2.5 to 10μM) reversibly antagonized responses to Q and K by up to 95% in a concentration - dependent manner whereas depolarizations evoked by L-aspartate and NMDA were reduced by a maximum of 18%. DNQX (5μM) caused parallel shifts in the log concentration - effect curves of Q and K to the right, giving mean (± s.e. mean, n = 7) dose ratios of 6.62 ± 0.89 and 5.10 ± 0.33, respectively, values which were significantly greater (Student t-test, $P<0.001$) than the corresponding dose ratios for L-aspartate and NMDA (1.31 ± 0.05 and 1.24 ± 0.07, respectively).

In a second series of experiments, transmission at the LOT-pyramidal cell synapse was monitored by recording the surface field potential known as the N-wave. Cumulative log concentration - effect curves were constructed for DNQX (1 to 10μM). DNQX caused a concentration - dependent reduction in the amplitude of the N-wave, the concentration producing a 50% reduction in amplitude being 2.92 ± 0.33μM (mean ± s.e. mean, n = 4). When repeated using slices perfused with 50 μM 2-amino-5-phosphonopentanoic acid, an antagonist selective for NMDA receptors, the concentration of DNQX required to reduce the amplitude of the N-wave by 50% was 3.59 ± 0.45 μM (mean ± s.e. mean, n = 4).

These results show that DNQX antagonises monosynaptic excitations in the olfactory cortex by a mechanism not involving NMDA receptors, thus supporting the original proposal (Collins, 1982) that receptors of the Q and/or K type mediate transmission at the LOT-pyramidal cell synapse. However, a presynaptic site of action of DNQX cannot be ruled out.

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EFFECT OF NON-NMDA EXCITATORY AMINO ACID RECEPTOR BLOCKADE ON HIPPOCAMPAL SLICE ICTAL EVENTS IN PHYSIOLOGICAL AND LOW Mg^{2+} MEDIUM

W.W. Anderson & E.J. Coan[1] (introduced by G.L. Collingridge), Department of Neurology and Molecular Neurobiology Laboratories, Medical College of Virginia, Richmond, VA, USA and [1]Department of Pharmacology, University of Bristol, Bristol, BS8 1TD.

Removal of extracellular Mg and electrical stimulation in physiological medium produce electrographic seizures (EGSs) in the rat hippocampal slice (Anderson et al. 1988). Adding the NMDA receptor blocker D-2-amino-5-phosphonovalerate (APV) to physiological medium blocks stimulus train induction of EGSs, but does not block EGS generation following induction (Stasheff et al. 1988). Adding APV to Mg-free (0-Mg) medium also does not block EGS generation, but does raise the threshold for seizure initiation and reduces EGS duration. The contribution of kainate/quisqualate receptor activation to seizure initiation and generation was tested by applying the antagonist 6-cyano-2,3-dihydroxy-7-nitro-quinoxaline (CNQX). Experiments were performed on hippocampal slices from young (21-33 day old) female Sprague-Dawley rats. Extracellular recording and stimulating electrodes were placed in stratum pyramidale and radiatum of CA3.

When 100 uM APV was added to 0-Mg + 2 uM baclofen (BAC), EGS duration was reduced and the threshold for EGS initiation increased substantially (Fig. 1B). When 10 uM CNQX was added to 0-Mg + BAC, EGS duration was reduced by half, the threshold for EGS initiation increased slightly, and clustered 'clonic' bursting was replaced by single bursts (Fig. 1C). When APV + CNQX were added to 0-Mg + BAC, EGSs were completely blocked (Fig. 1D). In contrast, when only 10 uM CNQX was added to physiological (0.9 mM Mg) medium following EGS induction, EGSs were completely blocked.

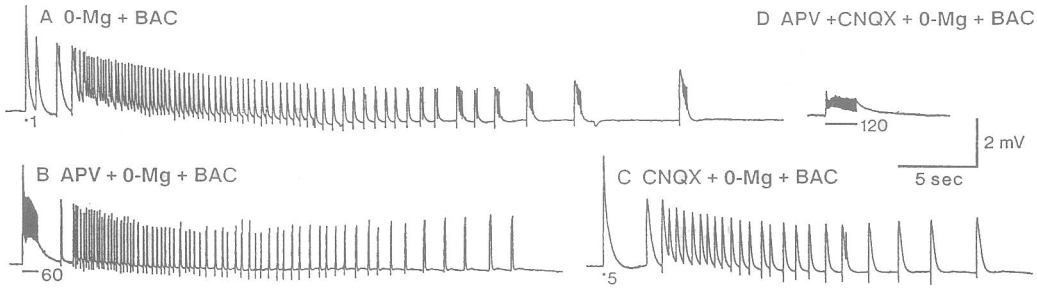

Fig. 1. The effect of 100 uM APV and 10 uM CNQX on electrographic seizures in 0-Mg + baclofen. The line and number under each record marks the number of stimulus pulses.

These results suggest that both NMDA and kainate/quisqualate receptor activated currents make substantial, although different, contributions to seizure generating currents in Mg-free medium, but in physiological Mg only kainate/quisqualate receptor activated currents substantially do. This is consistent with Mg blockade of the NMDA channel in physiological medium. NMDA, but not kainate/quisqualate, receptor activation also substantially lowers the threshold for EGS initiation.

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THE DISTRIBUTION OF HIGH-AFFINITY [^{3}H]-DEXTROMETHORPHAN BINDING SITES IN RAT BRAIN DIFFERS FROM THAT OF NMDA SITES

N.G. Bowery, R. Davey, M. Pelicano & F. Tortella, Dept of Pharmacology, The School of Pharmacy, 29/39 Brunswick Square, London & °Division of Neuropsychiatry, Walter Reed Army Institute of Research, Washington DC, USA.

Dextromethorphan (DM) is a non-opioid antitussive which exhibits significant anticonvulsant activity (Feeser et al 1988; Wong et al 1988). DM antagonises the response to N-methyl D-asparate in brain slices (Church et al 1985; Wong et al 1988) and acts as an anticonvulsant against NMDA-induced seizures in rats (Ferkany et al 1988). Additional data obtained in radiolabelled binding studies suggest that sites labelled with tritiated DM may relate to sigma sites since the rank order of potency of sigma ligands which displace ^{3}H-DM binding in guinea pig brain homogenates is similar to that for sites labelled with ^{3}H-3-(3-hydroxyphenyl)-N-(1-propyl)-piperidine ((+)PPP) and ^{3}H-(+)N-allyl-N-normetazocine ((+)SKF 10047) (Klein et al 1988). As a consequence we have performed experiments using quantitative receptor autoradiography to define the distribution of binding sites for ^{3}H-DM to make comparison with those of NMDA and PPP sites in rat brain.

Cryostat sections (10um) of Wistar rat brains were glass mounted and stored at -20°C. Prior to labelling thawed sections were incubated for 30 min in tris-HCl buffer (50mM, pH 7.4) at 20°C. Optimal binding was obtained by incubation with 8nM ^{3}H-dextromethorphan (70.8 Ci/mmole) in buffer solution at 20°C for 20 min. Sections were then rinsed in fresh solution (4°C) for 2x8 seconds and dried before apposition with LKB Ultrofilm for 4 weeks. Background binding was determined with unlabelled DM (10 mM). Specific binding was fully displaced by chlorpromazine (1 mM) but not by codeine (10 mM) which concurs with membrane binding data obtained by Craviso & Mussachio (1983).

The highest density of ^{3}H-DM sites occurred in the cerebellar molecular layer (10.9±2.1 nCi/mg tissue) with a much lower level in the granule cell layer. The density rank order determined from 4 sections from each of 4 rat brains was cerebellar molecular layer>thalamus>caudate putamen>cerebral cortex>hippocampus CA1>pons>cerebellar granule cell layer. This contrasts with the order for NMDA sites —hippocampus CA1>cerebral cortex>caudate putamen>thalamus>cerebellar granule cell layer>pons>cerebellar molecular layer and that of sites labelled with ^{3}H (+) PPP — pons>cerebellum granule layer>thalamus >cerebellum molecular layer>cerebral cortex>hippocampus radiatum>caudate putamen.

In conclusion we have demonstrated using receptor autoradiography the presence of binding sites for ^{3}H-DM in rat brain. Our data indicate that the distribution of ^{3}H-DM sites in rat brain bears no resemblance to that of NMDA or PPP sites and lend no support to the contention that DM acts directly on the NMDA receptor complex.

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THE EFFECTS OF NOVEL KAINATE/QUISQUALATE ANTAGONISTS AND PHORBOL ESTERS ON AMINO ACID-STIMULATED INOSITOL PHOSPHATE FORMATION

Philip P. Godfrey and Zahra Taghavi, Dept. of Clinical Pharmacology, Radcliffe Infirmary, Woodstock Road, OXFORD, OX2 6HE.

There is now good evidence that excitatory amino acids (EAA) can both stimulate and inhibit phosphoinositide turnover in a variety of neuronal systems (Godfrey et al, 1988; Sladeczek et al, 1988). There is concern, however, that these are real receptor-mediated responses since no selective antagonists have been identified (Godfrey et al, 1988). Here we have investigated the action of a number of novel EAA antagonists on both the stimulatory and inhibitory effects of EAA in cerebral cortex; the actions of protein kinase C effectors have also been examined since these are known to alter the functioning of inositide/G protein linked receptors.

Rat cortical slices were prepared and incubated as described previously (Godfrey et al, 1988). Slices were labelled with [^{3}H]-inositol for 45 min and then stimulated with agonist for 30 min; antagonist or phorbol ester was added 10 min prior to agonist. Incubations were stopped with chloroform/methanol (1:2) and inositol phosphates (IP) were separated by anion exchange chromatography.

As observed previously (Godfrey et al, 1988) both quisqualic acid (Quis) and ibotenic acid (Ibo) stimulated IP production in rat cortical slices. Quis (0.3 mM) stimulated the rapid formation of inositol bis, tris and tetrakis-phosphates, with a slower linear rise in the monophosphate which plateaued after 20 min. The responses to both Ibo (0.2 mM) and Quis (0.3 mM) were dose-dependently inhibited by phorbol dibutyrate (PDBU); the Ibo response was particularly sensitive to PDBU, with a 90% inhibition of the response at 1uM and an IC_{50} of 200 nM; Quis stimulated IP formation was less sensitive with a 50% inhibition observed at 10 uM PDBU. PMA (1 uM) and dioctanoylglycerol (30uM) also inhibited Ibo-stimulated IP formation though 4-B- phorbol (1-10 uM) was ineffective. The inhibition by PDBU was reversed with staurosporine (10 uM) and polymyxin B (10 uM), and both protein kinase C (PKC) inhibitors potentiated the Ibo response in the absence of PDBU. The putative Quis/kainate receptor antagonists 3-hydroxy-2-quinoxalic acid (HQ), 6,7-dichloro-3-hydroxy-2-quinoxalic acid (DHQ), 6,7-dinitroquinoxaline-2,3-dione (DNQX) and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) did not inhibit the responses to either Quis or Ibo at a concentration of 100 uM.

Kainate (100 uM) inhibited carbachol-stimulated IP formation by 80% (Godfrey et al, 1988); this inhibition could still be observed in tissue that was prestimulated with kainate for 10 min, followed by removal of kainate by washing, and subsequent stimulation with carbachol. The inhibitory action of kainate could be partially (20-30%) reversed by the Quis/kainate antagonists (100uM) and also by kyneurenic acid (1mM).

The data presented supports our hypothesis (Godfrey et al, 1988) that stimulation of IP formation by EAA is via a site distinct from the previously recognised EAA receptors; the inhibition of the responses to Ibo and Quis by activators of PKC approximates that observed for other agonists and suggests that a similar type of receptor may be linked to EAA. It seems likely that the inhibitory action of kainate is due to the excitotoxic effect of this class of neurotransmitter.

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ISOLATION OF FOUR COMPONENTS OF THE SYNAPTIC RESPONSE IN HIPPOCAMPAL CA1 PYRAMIDAL NEURONES

Ceri H. Davies, Stephen N. Davies & Graham L. Collingridge,
Department of Pharmacology, School of Medical Sciences,
University of Bristol, University Walk, Bristol, BS8 1TD.

The hippocampal slice has been used for many years as an experimental model in which to study synaptic transmission. We have been particularly interested in the response evoked in CA1 pyramidal neurones by stimulation of the Schaffer collateral-commissural tract (SCCT). With intracellular recording in standard magnesium containing medium this response comprises an excitatory amino acid mediated EPSP, and a two phase GABA mediated IPSP. Use of the following antagonists; 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10-20 μM) to block quisqualate/kainate receptors, D-2-amino-5-phosphonovalerate (APV, 20-40 μM) to block N-methyl-D-aspartate (NMDA) receptors, picrotoxin (100 μM) to block $GABA_A$ receptor gated chloride channels, and phaclofen (1 mM) to block $GABA_B$ receptors, combined with the appropriate siting of the stimulating electrode, has now allowed us to separate this intracellularly recorded response into four pharmacologically distinct components.

For example, with a stimulation electrode placed in the SCCT at some distance (> 0.5 mm) from the recording site, addition of CNQX to the recording medium considerably reduces both the EPSP and the polysynaptic IPSP and unmasks an APV-sensitive EPSP. At higher stimulus strengths this EPSP is curtailed by an IPSP. Pharmacological blockade of the IPSP could be used to leave a pure NMDA receptor-mediated EPSP.

With a stimulation electrode placed in the SCCT close to the recording site, addition of CNQX and APV blocks both components of the EPSP but leaves a distinct two-phase IPSP. This IPSP is blocked by tetrodotoxin and is believed to result from the direct stimulation of the axons or cell bodies of GABA-ergic neurones in the CA1 region. Selective antagonism of early or late phases of this IPSP can be achieved using picrotoxin or phaclofen, respectively.

Using these techniques it is possible to activate in isolation; a) the quisqualate/kainate receptor-mediated component of the EPSP, b) the unmasked NMDA receptor-mediated component of the EPSP, c) the $GABA_A$ receptor-mediated early phase of the IPSP, or d) the $GABA_B$ receptor-mediated late phase of the IPSP. This will allow more rigourous characterization of the physiological, biophysical and pharmacological properties of each of these components of the synaptic response.

This work was supported by the MRC. CHD is an A.J. Clarke Scholar.

A METHOD FOR MEASURING CHANGES IN AGONIST-INDUCED RESPONSES ASSOCIATED WITH LTP IN RAT HIPPOCAMPUS

Stephen N. Davies, Klaus G. Reymann[1] & Graham L. Collingridge. Department of Pharmacology, School of Medical Sciences, University of Bristol, University Walk, Bristol, BS8 1TD.

Using intracellular recording we have previously demonstrated a delayed increase in the sensitivity of CA1 pyramidal neurones to ionophoretically administered quisqualate following the induction of long-term potentiation (Collingridge & Lester, 1988). We now demonstrate a method for simplifying the measurement of this effect based on the use of focal DC potentials (Lambert et al., 1981).

An extracellular recording electrode is placed in stratum radiatum of the CA1 area and its position adjusted so as to maximise the field EPSP recorded in response to stimulation of the Schaffer collateral-commissural pathway. An ionophoretic pipette is then introduced close to the recording site and its position adjusted to achieve brisk DC potential changes in response to ionophoretic ejection of ∝-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid (AMPA). Ejection current (40-155 nA) and time (10-25 s) are set to evoke a submaximal response at 15 min intervals. Stable responses can be obtained for several (> 4) hours. These are depressed by 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX, 10 μM), but are unaffected by concentrations of cadmium (50-200 μM) which substantially reduce synaptic transmission.

Tetanization of the synaptic input (100 Hz for 1 s at double test intensity) on either side of the recording site (in order to maximise the number of tetanised synapses) usually results in immediate and sustained potentiation of the field EPSP but no initial change in the height of the AMPA-evoked potentials. Usually, however, the size of the AMPA response starts to increase 15-30 min post-tetanus and reaches a maximum after about 2 h (mean ± s.e.m. = 162 ± 25 % of control, n = 11).

This technique has a considerable advantage over intracellular methods in that it allows routine measurement of sensitivity changes over several hours.

[1]Permanent address: Institute of Neurobiology and Brain Research, Magdeburg, GDR. This work was supported by the MRC, the Wellcome Trust and the Academy of Sciences GDR.

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A GREASE-GAP METHOD FOR STUDYING LTP OVER LONG TIME PERIODS IN RAT HIPPOCAMPAL SLICES

J.F. Blake, M.W. Brown and G.L. Collingridge*, Departments of Anatomy and *Pharmacology, School of Medical Sciences, BRISTOL, BS8 1TD.

In rat hippocampal slices the phenomenon of long-term potentiation (LTP) is generally studied using either intra- or extra-cellular microelectrode techniques and can be recorded reliably for up to a maximum of about 8 h post-tetanus. In the method described here LTP can be studied in slices for approximately 1 day. Transverse rat hippocampal slices (400 um) were set up for recording at 26 ^{o}C with the alveus in a grease seal (Blake et al, 1988). The Schaffer collateral-commissural pathway was stimulated once every 30 s to evoke synaptic potentials, and agonists (in 2 ml aliquots) were added to the perfusion medium. This medium comprised (in mM); NaCl 124, $NaHCO_3$ 26, KCl 3, $CaCl_2$ 2, $MgSO_4$ 1 and D-glucose 10 (bubbled with 95% O_2 / 5% CO_2). LTP was induced by applying 5 tetani (each of 100 Hz for 0.5 s) at 30 s intervals.

Figure 1 illustrates an example where tetanic stimulation elicited LTP which lasted for at least 17 h. The slice was 7 h old when the tetani were delivered.

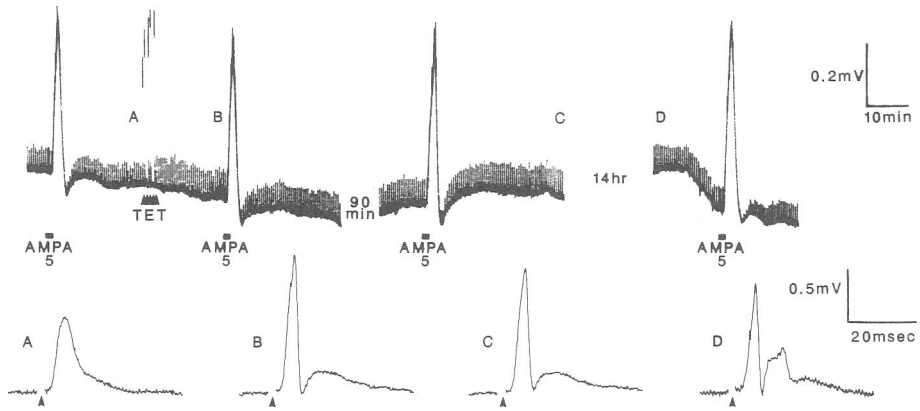

Figure 1. LTP lasting for 17 h in a hippocampal slice. Chart record shows depolarizations induced by AMPA (5 uM) and filtered versions of the synaptic response. At the position marked TET, 5 periods of tetanic stimulation (each comprising 100 Hz for 0.5 s) were delivered. Synaptic responses shown below are the averages of 5 consecutive records taken from the positions indicated on the trace. A = control, B = 15 min, C = 3 h, and D = 17 h post-tetanus.

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CALCIUM IN PITUITARY CELLS: MEASUREMENTS USING CONFOCAL MICROSCOPY AND WHOLE CELL PATCH CLAMPING

A.D.Randall, O.T.G.Jones, J.B.Chappell, S.L.Shorte, G.L.Collingridge* and J.G.Schofield. Department of Biochemistry and *Pharmacology,School of Medical Sciences, University of Bristol, University Walk, Bristol. BS8 1TD.

Intracellular ionic concentrations can be measured in single cells using dyes whose fluorescence is sensitive to changing ion concentrations (Tsien and Poenie, 1986). The optical properties of Fura-2 permit quantitative measurement of intracellular calcium concentrations, since the ratio of its fluorescence emissions on excitation by two alternate wavelengths is a function of the calcium concentration. Such studies utilise a fluoresence microscope equipped with a camera which is adapted to permit accurate quantification of emmitted light levels (see eg accompanying abstract).

An alternative approach is to use confocal scanning laser microscopy (Fine et al., 1988), in which a photomultiplier can produce digitally encoded fluorescence images of a discrete focal plane of living tissue with high definition. The recent development of a calcium sensitive dye compatible with the optical output of an argon laser, Fluo-3, has allowed the confocal microscope to be used to provide calcium dependent fluorescence images from cells.

Using these techniques it is therefore possible to probe changes in cell cytosolic calcium caused by both electrical and agonist stimulation. We will demonstrate, utilising the confocal microscope, the use of the calcium sensitive dye Fluo-3 to indicate changes in cytosolic calcium in secretory cells from the pituitary gland. We will also demonstrate the isolation of calcium currents using whole-cell patch clamp techniques, a major source of the increase in calcium ion concentration involved in the secretory response of these cells.

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Tsien, R.Y. & Poenie, M. (1986) Trends Biochem. Sci. 11,450-455.

SPATIALLY RESOLVED MEASUREMENT OF $[Ca]_i$ IN ANTERIOR PITUITARY CELLS

S.L. Shorte, J.B. Chappell, G.L. Collingridge*, J.G.Schofield and A.D. Randall, Departments of Biochemistry and *Pharmacology, School of Medical Sciences, University of Bristol, Bristol BS8 1TD, U.K.

The use of fluorescent calcium binding dyes such as Fura-2 in the determination of cytosolic free calcium ion concentration ([Ca]i) in individual living cells is now a well-established technique (Tsien and Poenie, 1986).

We recently reported the application of this technique to determine the changes in [Ca]i in single cells dispersed from bovine anterior pituitary gland upon application of known secretagogues such as TRH (Chappell et al 1988). In that investigation a photomultiplier was used to measure the fluorescence of individual cells loaded with Fura-2. This procedure has two major limitations, both of which arise from the non-spatially resolving nature of the photomultiplier, in that only one cell can be monitored at any time and it is impossible to observe localized changes within the cell. These disadvantages can be overcome by replacing the photomultiplier with a video camera (Tsien and Poenie, 1986). We have now used an intensified charge coupled device camera (ISIS Camera, Photonic Science, Tunbridge Wells, Kent) linked to an image-processing system (Magiscan, Joyce-Loebl, Tyne and Wear), to observe the dye-loaded cells. The inverted fluorescence microscope and its periphery remain as before.

This system can record successive frames from a field of cells and can subsequently calculate the fluorescence ratio or mean [Ca]i in individual cells within the field and the spatial distribution of this signal within each individual cell. The demonstration will show that the system can reveal heterogeneity both in the rates and magnitudes of the change in [Ca]i in pituitary cells in response to secretagogues such as nicotine, acetylcholine and TRH.

Tsien,R.Y. and Poenie,M. (1986) Trends Biochem. Sci. 11: 450-455.

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THE SEPARATION, DETECTION AND QUANTIFICATION OF NEUROACTIVE AND OTHER AMINO ACIDS USING A SENSITIVE HPLC METHOD

Lyn S. Mozley, Sarah J. Waller, Peter S. Harrison and Ian C. Kilpatrick, Dept. of Pharmacology, School of Medical Sciences, University Walk, Bristol, BS8 1TD

Amino acids subserve a number of roles in nervous tissue. Perhaps of special interest to pharmacologists and others are those amino acids that, on the basis of electrophysiological and neurochemical studies are considered to be neurotransmitters. For example, an excitatory neurotransmitter function has been ascribed to aspartate and glutamate whilst inhibitory neurotransmitters are considered to include 2-aminoethanesulphonic acid (taurine) and 4-aminobutanoic acid (GABA). Recently, the possibility that sulphinic and sulphonic acid analogues of aspartate and glutamate may be excitatory neurotransmitters in the central nervous system has been raised (Mewett *et al.*, 1983; Do *et al.*, 1986; Kilpatrick and Mozley, 1986) and a quantitative analysis of some of these latter compounds in spinal cord and brain tissue is presented elsewhere at this meeting (Waller *et al.*, 1989).

This demonstration will show how these and other amino acids can be selectively or simultaneously assayed with high sensitivity from extracts of microdissected brain tissue or in fractions collected from superfused brain slices.

The method relies on a Gilson HPLC system to perform the automatic pre-column conversion to and injection of *o*-phthalaldehyde/2-mercaptoethanol derivatives that are separated with a reverse-phase gradient. The amino acid derivatives are then detected fluorimetrically with a detection limit of some 300-500 fmol. Chromatogram analysis and quantification of peaks based on external and internal standards is achieved using a PC-based integrating package (Axxiom Model 747).

We thank the Wellcome Trust and the Medical Research Council for support.

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COMPUTER SIMULATION OF THE EFFECTS OF DRUGS ON NEUROTRANSMITTER RELEASE IN THE ENTERIC NERVOUS SYSTEM

D.G. Dewhurst & A.S. Meehan, Dept. Biomedical Sciences, Sheffield City Polytechnic, Pond St., Sheffield S1 1WB.

The use of microcomputers as a teaching aid in pharmacology has increased in recent years and a number of programs which simulate pharmacological experiments are available (Hughes 1982, 1987, 1988; Dewhurst et al. 1988). This package is a programmed learning exercise designed for undergraduates on a variety of courses e.g. medicine, pharmacolgy, pharmacy. It simulates the transmurally stimulated guinea-pig ileum preparation and is based upon a practical schedule developed and used in the Dept. of Physiology & Pharmacology, Nottingham University (Kendall 1988).

The program is written in Turbo Pascal 4 (Borland) and requires an IBM PC or compatible machine to run. It is easy to use, menu driven, guiding but not determining the students experimental approach to the preparation. The student may choose the frequency and magnitude of the stimulus voltage and select individual or combinations of drugs from a list. Results are displayed on the monitor and resemble those obtained on a scrolling chart recorder. Hard copy is available to those with a printer. Through the use of an accompanying booklet the student is required to:

i) determine whether morphine and clonidine act pre or post synaptically to inhibit transmural stimulation,

ii) examine the effects of naloxone and phentolamine on the inhibitory action of morphine and clonidine,

iii) demonstrate the inhibitory effect of high frequency transmural stimulation,

iv) examine the effect of naloxone and phentolamine on the inhibitory action of high frequency stimulation.

The completed booklet could provide the basis for assessed work.

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We would like to thank D.R. Tomlinson and S.J. Hill of Nottingham University for their assistance.

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A THERMAL STIMULUS DEVICE FOR EVALUATING ANTINOCICEPTIVE AGENTS IN THE CONSCIOUS UNRESTRAINED SHEEP BASED ON A BBC MICROCOMPUTER

I. Elliott, S. Ley, A. Livingston, R. Morris & A. Waterman, Departments of Pharmacology and Veterinary Surgery, University of Bristol, BS8 1TD.

We have previously described a technique for the evaluation of thermal threshold values in sheep (Nolan et al., 1987). This technique has been extensively modified and improved to allow a more reliable generation of the thermal ramp and to provide a visual demonstration of the ramp and cut-off. The basic requirements of the earlier device have been retained; namely that a maximum cut-off is included at a temperature which does not cause tissue damage, that the animal itself operates the normal cut-out by shaking its head or ear, that a manual override is included in case the animal fails to trigger the cut-out and that a failsafe circuit is included which switches off the heater circuit in the case of any component failure.

The apparatus consists of an earclip which is applied to the pinna of the sheep's ear, the clip contains a heater unit consisting of a resistor in a small copper tube, a thermocouple which is applied to the resistor and records the temperature and an inertia cut-out device which switches off the current to the resistor when activated. The thermocouple output is connected to a thermocouple amplifier which linearises the output and converts it into ^{o}C. The output is then connected to the Analogue input on a BBC microcomputer. The microcomputer reads and stores the temperature at time intervals determined by the software. The microcomputer also provides a digital output to drive a digital-to-analogue converter and a power amplifier which provides the ramp for heating the resistor in a linear manner, the rate of increase of the ramp may be varied from a menu in the software.

In addition the microcomputer provides a visual display of the temperature of the ramp and a display of the cut-out parameters, such as the cut-out time, cut-out temperature and experiment time and number. A storage and retrieval system allows the saving of this data on a floppy disc and subsequent hard copy on a printer.

This apparatus allows the recording of thermal threshold values in conscious unrestrained sheep and the evaluation of the effectiveness of various analgesic agents in raising these thresholds.

Nolan, A., Livingston, A., Morris, R. & Waterman, A. (1987) J. Pharm. Meth. 17, 39-49.

Supported by the Wellcome Trust.

MEASUREMENT OF BLOOD PRESSURE IN CONSCIOUS MARMOSETS

V. Worrall, B. Loveday, A.A. Oldham. Bioscience Department II, ICI Pharmaceuticals, Alderley Park, Macclesfield, Cheshire, SK10 4TG.

Methods of measuring blood pressure from conscious marmosets have been reported by others (for example, Hofbauer et al, 1983), but have required almost total restraint and administration of sedatives. The system used here is a modified version of the tether and harness system used for measuring blood pressure of unrestrained rats (for example, Russell et al, 1982).

Marmosets were prepared with an indwelling internal iliac arterial cannula, exteriorised at the back of the neck. Catheter patency could be maintained for several weeks. Animals were trained to wear a small back-pack which acted as an interface between the indwelling cannula and a small-diameter flexible tether containing a catheter for remote measurement of blood pressure. During recording sessions, animals were housed in a box which was of similar dimensions to the nesting box in their home cage (25x25x25cm). This method allowed the blood pressure of the marmosets to be measured without undue stress. Sedatives were unnecessary.

The demonstration will illustrate the equipment used and present the results of a study using the angiotensin-converting enzyme inhibitor captopril in this model, following sodium-depletion of the marmosets with low sodium diet and diuretic treatment.

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600P

A COMPUTER SIMULATION OF THE IN VIVO SUPERIOR CERVICAL GANGLION PREPARATION OF THE ANAESTHETISED CAT

D.G. Dewhurst & C.M. Howells, The Dept. of Biomedical Sciences, Sheffield City Polytechnic, Sheffield S1 1WB.

A number of computer simulations of pharmacological experiments are now available for use in undergraduate practical teaching (Hughes, 1982, 1987, 1988; Brown et al., 1988). These can be used to improve the preparation of students about to undertake the experiment, to replace the real experiment or as self-contained, student-centred learning packages. Here we describe an interactive CAL program based on a simulation of the in vivo superior cervical ganglion nictitating membrane preparation of the anaesthetised cat designed to teach the pharmacology of synaptic transmission at both the autonomic ganglion and the effector synapse.

The program, which is menu-driven, is written in Turbo Pascal 4 (Borland) for IBM-PC compatible machines and makes extensive use of graphics. Introductory sections provide sufficient detail of the pharmacology of transmission at each synapse for students to plan experiments. In these sections brief textual information is supported by high-resolution colour diagrams. The simulated preparation allows contractions of both nictitating membranes (NM) to be displayed simultaneously. Electrical stimulation to evoke a half maximal contraction can be applied to the preganglionic nerves.

The main part of the program allows students to administer different drugs to the preparation and to study their effects. No guidance is given, though more detailed information on any of the drugs is available within the package. Students can thus learn by discovery using their own initiative or they may be provided with a practical schedule to follow. Drugs to be investigated are selected from a comprehensive list which includes a randomized unknown. The student selects to administer the drug of choice, either to the ganglion or NM on one side only, in the absence of or during preganglionic nerve stimulation. Simulated results are derived from actual experimental data and are presented in high-resolution graphics on a continuously moving screen display to simulate a chart recorder. The displayed record may be output to a printer. The timescale is greatly speeded up so that results from experiments which may last several minutes can be displayed in a fraction of the time.

This work was supported by The Lord Dowding Fund (NAVS).

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A SOFTWARE SIMULATION OF THE PHYSIOLOGICAL RESPONSE TO GRADED EXERCISE IN MAN

D.G. Dewhurst & A.S. Meehan, The Dept. of Biomedical Sciences, Sheffield City Polytechnic, Pond St. Sheffield S1 1WB.

Computer simulations can be an effective means of providing students with physiological data in circumstances where, for a variety of reasons, it may be impossible for them to perform the experiments for themselves (Brown et al., 1988; Clarke, 1986). One such example would be the investigation of the physiological responses to graded exercise in man which requires expensive monitoring equipment lacking in most schools and colleges. A computer program, suitable for students at A-level and above, will be demonstrated which uses a mathematical model to generate typical cardio-respiratory responses of subjects performing graded exercise on a bicycle ergometer. The program, which is written in a combination of BBC BASIC and assembly language, is menu driven, interactive and makes use of mode 1 four-colour graphics.

A brief introduction leads to a screen display which prompts the student to input parameter values for their chosen subject (height, weight, gender, age, trained/untrained). Once defined, the subject's response to graded exercise can be investigated. The program will display traces of heart rate, minute ventilation and oxygen consumption which are presented on a scrolling display comparable to that of a chart recorder. The initial record is of the pre-exercise response and a keypress begins the experiment by applying a workload of 20 watts. The work load is incremented by 20 watts per minute until the subject is exhausted. Results are presented at up to ten times normal speed. A digital clock and one-minute time markers on the trace are provided. The experiment may be paused and restarted as required and a cross-hair cursor facility allows measurements to be made directly from the monitor screen.

It is also possible to monitor blood lactate concentration during the experiment. Students may simulate taking up to nine blood samples during the experiment (the heart rate trace is displayed) and the program records the time each is taken. Blood samples can then be 'analysed' using a simulated spectrophotometer which will give absorbance readings for either a range of lactate standards or the samples. Students can thus produce a graph of absorbance against lactate concentration using the standards provided and after measuring the absorbance of their samples can determine the lactate concentration from this graph. Blood lactate concentration can then be related to work load.

The model used enables students to generate realistic data from any chosen subject. By selecting different subjects the student can compare the cardio-respiratory performance of, for example, subjects of different ages, body size, sex and fitness.

Brown G.J., Dewhurst D.G., Meehan A.S (1988); J. Biol. Ed., 22(1), 19P.
Clarke, K.A. (1986); J. Physiol, 378, 8P.

DEVELOPMENT OF INTERACTIVE VIDEO TECHNIQUES FOR THE TRAINING OF PHARMACOLOGISTS

N.S. Hawkins, *J.A. Longstaffe and *M. Whittlestone (Introduced by R.H. Evans), Department of Pharmacology and *Department of Pathology, Medical School, University Walk, Bristol BS8 1TD.

Interactive video (IV) (Mercer et al, 1988) is essentially computer assisted learning enhanced with video pictures. Computer based tutorials (which interact with the student by giving information and requiring input) call up any of thousands of pictures (still or moving) generated from a videodisc. One side of a video disc may consist of up to 54,000 still frames or 36 minutes of moving sequences. Picture quality is high and speed of access from one picture on the disc to another is rapid. Because of the combination of video pictures, text and graphics on the same screen, students can be asked to point out salient features of the pictures as well as to give more conventional responses to multiple choice and text questions. Whereas computer assisted learning is exclusively "left brain" in nature IV technology, also allows the more effective "right brain" type learning to occur. IV takes automated learning far beyond the one paced, one way, "tape slide" tutorial and provides a new and flexible way of learning.

The material demonstrated consists of tutorials developed in Bristol based on a laser video disc provided by CIBA-GEIGY which shows effects of various drugs on animals. The material therefore has the additional feature of potentially reducing the use of animals in teaching.

The hardware consists of a BBC microcomputer in connected to a PHILIPS videodisc player, with appropriate interfaces (ABBEY overlay and COX genlock). The tutorials have been developed using an authoring language (VIPA) which has been specifically developed for writing IV tutorials (Harkin et al, 1986). VIPA allows the rapid development of IV tutorials by people with limited experience of computers.

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THE USE OF HYPERCARD AND THE APPLE MACINTOSH MICROCOMPUTER TO PRODUCE INTERACTIVE CAL PROGRAMS

D.G. Dewhurst, A.S. Meehan & M. Quentin-Baxter, The Dept. of Biomedical Sciences, Sheffield City Polytechnic, Pond St., Sheffield S1 1WB.

A number of computer simulations of pharmacological and physiological experiments are available. These are usually written in high-level programing languages such as BASIC, Pascal, Fortran or C and are difficult or impossible for non-programers to modify.

Authoring languages have been developed which can help the non-expert develop teaching packages but even these can be very involved and are often just higher level programming languages. For many teachers, a simple to use system for rapidly creating computer aided learning material would be attractive.

Apple Macintosh supply free with all new Macintosh computers a copy of HyperCard, an extremely easy to use multimedia software package incorporating high quality graphics, text, sound and (limited) animation.

Because HyperCard provides an object-orientated programing environment which doesn't require the use of a language it can be learnt in a matter of hours. For very complex presentations HyperCard has it's own English-like authoring language which can be further extended using Pascal or C but this need never concern most developers.

As HyperCard supports highly interactive text and graphics facilities it is an ideal medium for portraying information and testing students.

This demonstration will illustrate a program on Nerve Physiology previously demonstrated to this society on a BBC microcomputer (Dewhurst et al., 1986) which has been implemented in HyperCard. To highlight the ease with which HyperCard can be used members will be invited to make their own modifications to the package during the demonstration.

This work is supported by the Lord Dowding Fund (NAVS).

Dewhurst D.G., Meehan A.S., (1986); Br. J. Pharmac. 89, 882P.

SOME MICROCOMPUTING AIDS IN TEACHING AND DATA HANDLING

R.B. Barlow, Department of Pharmacology, Medical School, University Walk, Bristol, BS8 1TD.

The demonstration shows:

1. A set of programs for teaching statistics.
2. A set of programs for making the statistical tests referred to.
3. Multifit programs for fitting data to a wide variety of nonlinear expressions used in enzyme kinetics, ligand-binding studies, dose-response curves and experiments on drug metabolism.
4. The current version of "Molecular graphics with a microcomputer and a mouse" (Barlow, 1987), which gives rapid access to over 400 drug structures.

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BUSPIRONE AND GEPIRONE ANTAGONISE THE ANTIDEPRESSANT-LIKE EFFECT OF 8-OH-DPAT IN A PUTATIVE MODEL OF DEPRESSION IN MICE

K.F. Martin, L.J. Hutchins, J. Gosden, G.P. Luscombe and W.R. Buckett, Research Department, The Boots Company PLC., Nottingham, NG2 3AA.

COMMUNICATIONS

The author who intended to present the work is indicated by an asterisk (*) on the abstract

369P COLQUHOUN L, HOLDEN-DYE L* & WALKER AJ
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370P REGGIANI A*, GAVIRAGHI G & CESERANI R
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372P ROMANELLI L*, RATTI E, GAVIRAGHI G, CESERANI R & BOWERY NG
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373P LONG SK & EVANS RH*
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374P CONNICK JH*, CARLA V, MORONI F & STONE TW
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376P JONES MG*, YULIANG L, LODGE D & MILLAR JA
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382P DEXTER DT*, CARAYON A, JENNER P, AGID F, AGID Y & MARSDEN CD
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383P DE PAERMENTIER F*, CHEETHAM SC, CROMPTON MR & HORTON RW
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385P GLEITER CH*, WOZNIAK KM, COSTELLO MJ, NUTT DJ & DE JONG J
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398P MAIDMENT NT*, BRUMBAUGH DR, ERDELYI E, RUDOLPH VD, BARCHAS JD & EVANS CJ
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UNIVERSITY OF BRISTOL

5–7th April