EFFECTS OF AROCLOR 1254 ON IMMATURE RATS FOLLOWING EXPOSURE OF THE MOTHER EITHER PRIOR TO MATING OR DURING LACTATION

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Aroclor 1254 is a commercial mixture of polychlorinated biphenyls (PCB's). Toxicity of at least some of the 209 PCB congeners has been documented, largely associated with reproduction, carcinogenicity and immunotoxicity. Due to their lipophilic nature, PCB residues persist in body tissues and are believed to be mobilised along with fat. Prime times of fat mobilisation include pregnancy and lactation thus exposing the fetus and neonate. We have examined the effect of Aroclor 1254 on immature rats following pretreatment of their mothers either prior to mating or during lactation.

Female Wistar albino rats were used. One group (Group A; 200-250 g) were dosed weekly with Aroclor 1254 (20 mg/ml;30 mg/kg) by mouth for 6 weeks. The rats were mated one week following the final dose and the resulting pups allowed to suckle normally for 21 days when they were sacrificed. The second group (Group B) were dosed following commencement of lactation at a dose of 30 mg/kg twice weekly by mouth for 3 weeks. The pups were again allowed to suckle normally during this period and sacrificed at 21 days. For both groups, control animals were similarly treated substituting the Aroclor diluent, olive oil, for the Aroclor. Pups from each group were examined amongst other things for organ weights, liver aniline hydroxylase activity, and liver protein concentration.

There were no significant differences throughout the experiments in maternal body weights between the treatments and control groups. Litter size was not affected by either dosing regime, but total litter weights tended to be greater in the group dosed prior to mating and lower in the group dosed during suckling (compared to their controls). However, in both cases the differences just failed to reach significance. Total liver weight in female pups at 21 days (1.95 $\pm$ 0.24 g;n=8; mean $\pm$ sd) showed a significant increase over control pups (1.29 $\pm$ 0.08 g;n=8; $p<0.001$) when Aroclor was administered during lactation but no difference was observed if treatment was prior to mating. Liver microsomal protein was greater ($p<0.01$) in rats whose mothers were treated during the nursing period (28.73 $\pm$ 3.77 mg/g liver;n=4) compared to the control group (17.53 $\pm$ 3.77;n=4) although no change was observed in Group A. Aniline hydroxylase activity tended to be greater in both treatment groups although again significance was not achieved.

Both groups received the same total dose of Aroclor 1254 but significant changes in liver weight and protein concentration were only observed in pups where treatment had occured during suckling rather than prior to mating. In Group A, transfer could have occured either during gestation and/or lactation, whilst in group B, maternal transfer was via the milk. Since there was apparently a greater effect in the latter group, it may be concluded that these offspring received a greater dose of Aroclor 1254 when it was administered during the nursing period, or, it was transfered at a more susceptible period in rat development. No gross teratological effects were observed in the litters of either treatment groups.

IMMUNE COMPLEX-MEDIATED COLITIS IN MICE

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An immune complex (IC)-mediated colitis was induced in rabbits by Hodgson et al (1978), using the Auer technique. Immune complex was injected i.v. concomitant with intra-rectal instillation of dilute formalin as a topical irritant to cause localized deposition of the IC. We now report studies on the colitis induced by this means in mice.

Groups of 6 male BKA mice (20-30g) were used. Formalin (1%) in distilled water, was administered by intra-rectal instillation by insertion of a Trochar needle to a depth of 1.5cm. Immune complex, prepared using human serum albumin (HSA) (0.6mg/ml) and rabbit anti-HSA (22mg/ml of IgG) re-dissolved in excess antigen, was injected i.v. In time-course studies, animals were killed at various time intervals for assessment. Control animals received saline instead of the agent under test. Change in tissue water content, expressed as a percentage of tissue wet weight, was used to monitor colonic oedema. Colonic sections were stored in phosphate buffered formalin for subsequent "blind" assessment of histology by a clinical pathologist.

The increase in oedema following intra-rectal instillation of 1% formalin peaked at 3h after administration and had disappeared by 24h. Concentrations of formalin below 1% gave a submaximal response. When IC was injected i.v. at the 3h peak of the formalin response, a secondary oedema response was induced which peaked at 5 days after administration and had returned to healthy control levels after 10 days. At the peak of the IC response, all mice exhibited diarrhoea. The colonic tissues were erythematous and visibly oedematous. Histological examination showed the presence of polymorphs in the mucosa, goblet cell depletion and crypt abcess formation. The histological signs of inflammation commenced at 3 days and peaked at 5 days. Varying the time interval between formalin instillation and IC injection showed that the optimal time for the injection of IC was 3h following formalin instillation. The response was prolonged, but the severity of the response was not increased by giving a second i.v. injection of IC at the 5 day peak of the IC response.

The model was consistent and reproducible and the histology and pathology showed features similar to human acute colitis. The IC-mediated colitis in mice thus merits further evaluation as a model for the assessment of anti-colitic drugs.

LPW was supported by an SERC CASE studentship.

Hodgson, HJF, Potter, BJ, Skinner, J & Jewell, DP (1978) Gut, 19, 225-232

SPECIFIC ANTAGONISTS FOR BRADYKININ

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By incorporation of ß-(2-thienyl)-L-alanine (Thi), D-phenylalanine (DPhe) and L-4-hydroxyproline (Hyp) into the sequence of bradykinin and adding basic alpha-aminoacids like D-arginine or lysine (Lys) to the N-terminus of these peptides, Vavrek and Stewart (1985) synthesized peptides with antagonistic activity to bradykinin on several intestinal and vascular smooth muscle preparations. The antagonistic peptide B4310 (Lys-Lys-3-Hyp-5,8-Thi-7-DPhe-bradykinin) was tested on the following effects of bradykinin:

1. B4310 reduced the bradykinin-induced contraction of the rat uterus (pA_2 = 7.24).

2. Relaxation of the rat duodenum induced by bradykinin was inhibited by B4310 (pA_2 = 7.3).

3. Plasma protein extravasation into the rabbit skin, induced by intracutaneous injections of bradykinin (1.6 nmol), was reduced significantly when bradykinin was injected simultaneously with B4310 (7 to 60 nmol).

4. B4310 (5 to 500 nM) antagonized the bradykinin-induced venoconstriction in the isolated perfused rabbit ear in a dose-dependent manner. It had no effect on the arterial vasoconstriction induced by angiotensin II.

5. The bradykinin-induced release of prostaglandin E_2 from the isolated perfused rabbit ear (Lembeck et al., 1976) was reduced by 63% under infusion of B4310 (800 nM).

6. The bradykinin-induced stimulation of nociceptors in the vascular bed of the rabbit ear (Juan & Lembeck, 1974) was inhibited by an i.a. infusion of B4310 (50 and 500 nM) into the ear. The antagonism was dose-dependent, reversible and indicates an interaction on bradykinin receptors. B4310 did not antagonize acetylcholine-induced nociceptor stimulation.

7. The contraction of the rabbit iris sphincter muscle, which is mediated by substance P release due to stimulation of primary afferent trigeminal neurones by bradykinin, was antagonized dose-dependently by B4310 (pA_2 = 7.6).

It is concluded that B4310 inhibits specifically different actions of bradykinin which are related to its possible pathophysiological role.

Juan, H. & Lembeck, F. (1974) Naunyn-Schmiedebergs Arch. Pharmac. 283, 151-164.
Lembeck, F., Popper, H. & Juan, H. (1976) Naunyn-Schmiedebergs Arch. Pharmac. 294, 69-73.
Vavrek, R.J. & Stewart, J.M. (1985) Peptides 6, 161-164.

RENAL KALLIKREIN RELEASE FROM 3 SPECIES: THE EFFECT OF ARGININE VASOPRESSIN AND DESAMINO-D-ARGININE VASOPRESSIN

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Previous studies have shown that during the in vitro incubation of rat cortical kidney slices both active (AK) and inactive (IK) kallikrein are secreted into the incubation medium (Nustad et al, 1975) and the release of AK is enhanced by arginine vasopressin (AVP) in a static incubation system (Lauar et al, 1982).

The aim of the current study was to (i) identify the ratio of AK:IK released from rat, monkey and human cortical kidney slices, and (ii) to compare the effect on kallikrein release of AVP and the analogue desamino-D-arginine vasopressin (DDAVP) which is devoid of pressor activity.

Studies were carried out on kidney from 6 PVG rats, 4 cynomolgus monkeys and 3 humans; the latter was macroscopically normal tissue from kidneys with a nephroma. Cortical slices approx. 150 u were cut with a Macilwain chopper, and continuously perfused in holders with Krebs buffer (pH 7.4 aerated with 95% O_2) at a constant flow rate of 0.42 ml/min and a temperature of 37°C. Slices in four holders were simultaneously perfused for 1 hour to obtain a steady base line release of kallikrein. Four 15 min control perfusate collections were subsequently obtained from each holder. Two holders were then perfused with 4 x 10^{-8} M AVP and DDAVP in 0.9% saline solution respectively while the remaining holders acted as controls. A further four 15 min perfusate collections were obtained. AK was assayed using the chromogenic substrate H-D-Val-Arg-PNA (S2266) and IK following trypsin activation.

Kallikrein release by the kidney was almost completely in the IK form. There was no significant difference (at the 1% level) in the proportion of AK:IK in the 3 species (rat 5:95, monkey 3:97, human 3:97). The human kidney produced significantly more IK than the monkey and rat slices per unit wet weight ($p < 0.005$). Slices from the monkey and rat kidneys produced similar amounts. Both AVP and DDAVP produced a significant increase in IK release, to 191 ± 34% ($p < 0.002$) and 184 ± 34% ($p < 0.001$) respectively from rat kidney, compared to control perfusions, with rapid return to pre-stimulated levels. A similar response was found in the human and monkey tissue (monkey AVP response 175 ± 40%, DDAVP response 154 ± 21%, human AVP response 241 ± 64%, DDAVP response 164 ± 37%). There were no significant differences between the AVP and DDAVP responses in any of the 3 species.

The basal levels of AK released from slices of all 3 species were very low. AVP and DDAVP did not cause any significant change in AK in contrast to previous observations on rat kidney slices after more prolonged stimulation with AVP (Lauar et al, 1982).

This work was supported by a grant from the Wessex Regional Health Authority.

Nustad, K., Vaaje, K. (1975). Synthesis of kallikrein by rat kidney slices. Br. J. Pharmac. 53, 229-234.
Lauar, N., Shacklady, M., Bhoola, K.D. (1982). Factors influencing the in vitro release of renal kallikrein. Agents & Actions 9 (Suppl.), 545-552.

NEGATIVE FEEDBACK REGULATION OF CCK-8 AND MUSCARINIC RECEPTOR-MEDIATED PHOSPHOINOSITIDE METABOLISM IN PITUITARY CLONAL CELLS

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Recently, we have demonstrated the presence of cholecystokinin (CCK-8) and muscarinic cholinergic receptors in the human embryonic pituitary cell line Flow 9000. These two types of receptor both activated phosphoinositide (PI) turnover. Phorbol esters are tumour promotors which mimic the action of diacylglycerol (DAG) in activating protein kinase C (PKC) and are useful tools in elucidating the role of PKC in transmembrane signallings. In the present study, we have investigated the effects of phorbol esters on CCK-8 and acetylcholine (ACh) stimulation of inositol phosphate formation.

When preincubated with [^{3}H]inositol-labelled Flow 9000 cells, the active tumour-promotor, 4β-phorbol 12β-myristate 13α-acetate (TPA, 1uM, $_3$10min) produced a dose-dependent inhibition of CCK-8 and ACh-induced [^{3}H]InsP production while it had no effect on its own. The Ki value of TPA on CCK-8 stimulation is 1.9 $\pm$ 0.3nM (n=3) which is in accord with the nanomolar Kd value for TPA binding to PKC. Maximal inhibition by TPA (10uM) on CCK-8 (1nM) and ACh (40uM) stimulation of [^{3}H]InsP accumulation were 60% and 92% respectively. In contrast, the weak tumour-promoting agent, phorbol 13,20-diacetate (PDA) had no significant effect on either CCK-8 and ACh stimulation supporting the specificity of the TPA effect. One possible mechanism of the phorbol action is via protein phosphorylation as a consequence of PKC activation. The two possible sites of action are the receptor and the coupling protein (Np) involved in PI hydrolysis. The latter possibility is unlikely since GTP[S]-induced [^{3}H]InsP formation in saponin-permeabilized Flow 9000 cells was not affected by TPA pretreatment. One intriguing observation in this study was that the TPA inhibition on PI turnover was much higher in the case of ACh stimulation. This might be due to different degrees of phosphorylation of the two receptors by PKC.

These results suggest that both CCK-8 and muscarinic receptors which are linked to the activation of phosphoinositidase C and PKC are subjected to feedback regulation via the latter enzyme.

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μ AND δ, BUT NOT K, OPIOID AGONISTS DEPRESS SYNAPTIC TRANSMISSION IN THE MOUSE HYPOGASTRIC GANGLION

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Opioids depress both neuro-effector transmission in the vas deferens (Henderson et al., 1972) and synaptic transmission in the hypogastric ganglion (Henderson and Rogers, 1987) of the mouse. The present experiments were undertaken to determine the opioid receptor subtype mediating the depression of synaptic transmission in the hypogastric ganglion.

Mice (DBA I/1A strain) were killed by cervical dislocation. The hypogastric ganglion was disected out and pinned to the base of a recording chamber lined with silicon resin. The preparation was superfused with oxygenated Krebs solution at 37°C and drugs were applied in known concentrations by addition to the superfusate. Acetylcholine was applied by pressure ejection from a micro-pipette positioned above the ganglion. Intracellular recordings were made from ganglion cells with microelectrodes filled with 3M KCl. Excitatory post-synaptic potentials (epsps) were evoked by focal stimulation of preganglionic fibres. These epsps were nicotinic in origin, all-or-nothing, and suprathreshold for action potential initiation.

The μ selective opioid peptide, [D-Ala^2, NMePhe^4,Gly-ol^5]enkephalin (DAGO, 300nM-3μM, n=22) and the δ selective opioid peptide, [D-Ser^2]Leu enkephalin Threonine (DSLET, 3nM-1μM, n=29) depressed the amplitude of the epsp in a concentration-dependent manner. The time course of effect and maximum depression being similar for each drug. In contrast, the κ selective opioid U50488 (1μM, n=5) did not affect the epsp. Over the concentration range which depressed the epsp DAGO and DSLET were without effect on the resting membrane potential or input resistance of the post-synaptic neurone or the shape of the post-synaptic action potential. Also DAGO (1μM, n=5) and DSLET (1μM, n=6) did not depress the nicotinic response to acetylcholine evoked in the presence of atropine (1μM).

In 20 cells DSLET (100nM) and DAGO (300nM) were applied in succession. In each cell, the epsp was depressed by both agonists. However, the depression of the epsp by DSLET was prevented by prior exposure to the δ selective antagonist, ICI 174864 (300nM) whereas the depression by DAGO was unaffected (n=5). The depression of the epsp by DAGO and DSLET was prevented by naloxone (100nM-1μM, n=6).

Thus both μ- and δ-opioid receptor activation appears to depress the same synaptic input to neurones in the mouse hypogastric ganglion.

HENDERSON, G., HUGHES, J. & KOSTERLITZ, H.W. (1972) Br.J.Pharmac. 46, 764-766
HENDERSON, G. & ROGERS, H. (1987) J.Physiol., (submitted)

PD117302: A SELECTIVE AGONIST AT THE κ OPIOID RECEPTOR

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Kappa opioid agonists are potent analgesics and, in contrast to agents acting at mu receptors, have minimal addiction liability, respiratory depressant and constipating properties. The development of kappa-selective agonists as safe and effective analgesics is therefore being extensively investigated. Recently, a novel nonpeptide kappa opioid compound, (±) trans-N-methyl-N-[2-(1-pyrrolidinyl)-cyclohexyl]benzo[b]thiophene-4-acetamide, PD117302, has been synthesised (Clark et al. 1987) and shown to have potent antinociceptive activity in the rat,guinea pig and mouse (Hill et al. 1987). The compound has now been tritium labelled and its in vitro properties are described in this abstract.

Specific [^{3}H]PD117302 binding to guinea pig brain homogenates reached equilibrium within 20 min at 4°C, was saturable and reversible and appeared to interact with both a high-affinity (Kd = 2.7±0.6nM; Bmax = 3.4±0.9 pmol/g wet weight, n = 6) and a low-affinity (Kd = 1.0 uM; Bmax = 370 pmol/g wet weight) binding site. Binding of [^{3}H]PD117302 to the high-affinity site was competitively inhibited (Ki values in parentheses) by unlabelled PD117302 (2.4±0.5nM), by the standard kappa agonists, EKC (0.5±0.1nM) and bremazocine (0.15±0.04nM) but not by the mu- and delta- selective ligands DAGO (>1uM) and DPDPE (>1uM). Unlabelled PD117302 had a low affinity for both the [^{3}H]DAGO mu binding site (Ki = 410nM) and the [^{3}H]SKF 10047 sigma binding site (Ki = 1.8uM). Binding of [^{3}H]PD117302 to the low affinity site was not inhibited by either naloxone (>10uM) or SKF 10047 (>10uM).

PD117302 produced a concentration-dependent inhibition of the contractions induced by electrical stimulation of the guinea pig ileum (IC50 = 1.07±0.11 nM; n = 41) and the rabbit vas deferens (IC_{50} = 44.5±14.1nM; n = 3). The effect of PD117302 in the ileum was naloxone reversible (pA_2 = 7.78).

Quantitative autoradiographic data showed that [^{3}H]PD117302 binding sites were heterogeneously distributed in the central and peripheral tissues of the guinea pig. In particular,in the CNS a high density of binding sites was found in the striatum, n.accumbens, globus pallidus, cortical layers V and VI, s.nigra and the molecular layers of the hippocampus and cerebellum. In peripheral tissues, the highest density of binding sites was located in the kidney and adrenal cortex. This relative distribution of [^{3}H]PD117302 sites was similar to that of kappa receptors determined with other peptide and nonpeptide radioligands (Clark et al. 1986; Sharif et al. 1986).

In conclusion, these results suggest that PD117302 is a selective high affinity kappa-agonist in guinea-pig tissues whose autoradiographic distribution of binding sites resembles that of kappa opioid receptors determined with alternative radioligands.

Clark, C.R. et al. (1986). BPS London Meeting, December 1986.
Clark, C.R. et al. (1987). J. Med. Chem. (in the press).
Hill, R.G. et al. (1987). This meeting.
Sharif, N.A. et al. (1986). Biochem. Soc. Trans. 14, 688-690.

FORMATION OF [LEU^5] ENKEPHALIN FROM DYNORPHIN(1-8) BY RAT SPINAL CORD IN VITRO

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The endogenous peptide dynorphin(1-8) has some selectivity for the kappa-opioid binding site (Corbett et al, 1982). Since the spinal cord is an important site for analgesia and contains a high proportion of kappa-sites (Traynor et al, 1982) the pharmacology of dynorphin(1-8), and related dynorphins, after i.t. administration has been the subject of several investigations (e.g. Jhamandas et al, 1986). However the dynorphins are C-terminal extensions of [Leu^5]enkephalin and could conceivably be cleaved in vivo to the delta-preferring pentapeptide (Gillan et al, 1985; Griffiths et al, 1983; Miller et al, 1985). In this study we have investigated the breakdown of [^{3}H]dynorphin(1-8) by rat spinal cord tissue in vitro.

Lumbo-sacral spinal cord slices (0.5mm, 10mg) from male Wistar rats (250g) were incubated with [^{3}H]dynorphin(1-8) (10nM) in Hepes buffered Krebs solution at 37^{o}C, in the presence or absence of the peptidase inhibitors bestatin (10μM), thiorphan (0.3μM), captopril (10μM) and L-leucyl-L-leucine (2mM). Phosphoric acid (50mM) was added and supernatants stored at -20^{o}C prior to HPLC separation of the [^{3}H] metabolites using a C_{18} reverse phase column. Fractions corresponding to marker peptide standards were collected and counted for radioactivity.

[^{3}H]Dynorphin(1-8) present in the supernatant was degraded in a time-dependent manner to 2.42±1.30% (n=5) of recovered metabolites after 20min exposure to spinal cord slices. The major metabolic products appearing in the supernatant, accounting for 81.40±4.09%, resulted from removal of the N-terminus. Radioactivity co-eluting with the [Leu^5]enkephalin marker represented 11.01±3.23% of the recovered [^{3}H]metabolites and the tetrapeptide TyrGlyGlyPhe 3.54±1.77%. When incubations were performed in the presence of peptidase inhibitors [^{3}H]dynorphin(1-8) was still recovered as a minor fraction (16.16±4.16%, n=5). In contrast the level of [^{3}H][Leu^5]enkephalin was increased to 60.50±2.21% of the total recovered metabolites. [^{3}H]TyrGlyGlyPhe (14.03±2.03%) was present but [^{3}H]tyrosine accounted for only 9.59±2.16%. In both the presence and absence of peptidase inhibitors the [^{3}H][Leu^5]enkephalin-Arg^6 and -Arg^6-Arg^7 fragments represented less than 2%. It is important to note that the study has only measured [^{3}H] products present in the supernatant and takes no account of [^{3}H] material bound to the spinal cord tissue which may be more stable to peptidases (Gillan et al, 1985).

The results suggest that dynorphin(1-8) is cleaved at the Leu^5-Arg^6 bond and that this can be readily observed in the presence of peptidase inhibitors which stabilise the C- and N-termini. A probable candidate enzyme for this cleavage is the metalloendopeptidase EC 3.4.24.15 described by Orlowsky and colleagues (Chu and Orlowsky, 1985). Additionally the results emphasise that care should be exercised in the interpretation of data using dynorphin(1-8). Future experiments should clarify how the present findings relate to metabolism in vivo and to the physiological role of dynorphin(1-8).

Chu, T.G. & Orlowski, M. (1985) Endocrinology 116, 1418
Corbett, A.D. et al (1982) Nature 299, 79
Gillan, M.C.G. et al (1985) J. Neurochem. 45, 1034
Griffiths, E.C. et al (1983) J. Physiol. 343, 117P
Jhamandas, K. et al (1986) Can. J. Physiol. Pharmac. 64, 263
Miller, L. et al (1985) Eur. J. Pharmac. 116, 159
Traynor, J.R. et al (1982) Life Sci. 31, 1377

ATP-SENSITIVE CHANNELS IN NEONATAL RAT CULTURED NEURONES

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Potassium selective channels that can be inhibited by intracellular ATP have been reported in cardiac muscle cells (Noma, 1983), pancreatic β-cells (Cook and Hales, 1984) and skeletal muscle cells (Spruce et al, 1985). Recently we have shown that a calcium-activated non-selective cation channel in a rat insulinoma cell line can also be inhibited by ATP (Ashford et al, 1986). We now report preliminary results of an investigation into whether such channels exist in neuronal cells.

Cultures were obtained by mechanical and enzymatic dissociation of cerebral cortices or cerebella from 2-7 day old rats. Cortical neurones and cerebellar granule cells were identified on the basis of appearance (phase-bright and process bearing) and by positive neurofilament immunostaining. Single channel currents were recorded from inside-out membrane patches. The patch electrode was filled with either a K^+ - or (more commonly) a Na^+ - rich extracellular solution and the bath solution was a K^+-rich intracellular fluid containing 0.9 mM $CaCl_2$ and 1.0 mM EGTA resulting in a free calcium concentration of 10^{-6} M.

In patches obtained from cerebral cortex cultures and bathed in asymmetric cation solutions a number of different types of K^+-selective channel were observed. Only one of these was found to be inhibited by bath-applied ATP and it occurred in only 4 out of 34 membrane patches. This K^+-channel had a conductance of approximately 60-70 pS at membrane potentials around 0mV and ATP (2-10 mM) produced long closed periods in the single channel activity. A more commonly observed channel (18 out of 34 patches) in these cells was one that was equally permeable to Na^+ and K^+ ions. This non-selective channel displayed a pronounced rectification in the current-voltage relationship. The single channel conductance for outward currents at positive membrane potentials, was 58.1±2.0 pS and for inward currents, at negative membrane potentials, was 18.1±0.7 pS (mean ± s.e. mean; N = 6). ATP (1-3 mM) induced a "flickering" type of block of this channel which resulted in a reduction of the open state probability. Similar actions of ATP on a K^+-selective and non-selective channel were also observed in patches obtained from cerebellar granule cells. All effects of ATP were reversible on wash.

Our results indicate that ATP-sensitive K^+ channels do exist in certain cultured neuronal cells but are probably few in number. In addition we have discovered a novel ATP-sensitive non-selective channel.

Ashford, M.L.J. et al (1986) J.Physiol. 381, 116P
Cook, D.L. & Hales, C.N. (1984) Nature 311, 271-273
Noma, A. (1983) Nature 305, 147-148
Spruce, A.E. et al (1985) Nature 316, 736-738

RECOVERY OF MUSCARINIC RECEPTORS IN RAT BRAIN REAGGREGATE CULTURES AFTER IRREVERSIBLE BLOCKADE BY ETHYLCHOLINE MUSTARD AZIRIDINIUM

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Assayed five days after intrahippocampal injection of ethylcholine mustard aziridinium (ECMA) in rats (2 nmol injected in 2 µl) local choline transport and choline acetyltransferase (ChAT) activities were 77% and 58% reduced, but muscarinic receptor binding was unchanged (Fisher & Hanin, 1986). Similar results were obtained 7 days after intracerebroventricular (i.c.v.) injection of ECMA (lateral ventricles, 3 nmol/3 µl/side; Leventer et al, 1985). We have previously reported that ECMA causes rapid loss of ChAT (38%, 2h) in foetal rat brain reaggregate cultures (final concentration 12.5-50 µM; Atterwill et al, 1986; Pillar et al, 1987) followed by additional longer-term loss (total : 60-80%, 3-5 days). We therefore investigated muscarinic receptor binding in cultures similarly treated with ECMA, and in membrane preparations from brain of adult rats.

Cultures were prepared in foetal calf serum-supplemented medium from 16-17 day rat foetuses as previously described (Atterwill et al, 1984) . ECMA was freshly prepared (Pillar et al, 1987) and added directly to the cultures on the ninth and twelfth day in vitro (9 or 12 DIV) final concentrations 12.5, 25 or 50 µM. Aggregates were harvested by sedimentation, washed and homogenised in Na-phosphate buffer, pH 7.0. Membranes from cerebral cortex of adult male Wistar rats were prepared similarly, washed three times before use by centrifugation, then reacted with ECMA in culture medium or Na-phosphate buffer. Samples were assayed for ^{3}H-quinuclidinyl benzilate (QNB) binding (2 nM, 0.015 µCi) using atropine to define specific binding.

Table 1 : Inhibition of QNB binding in cultures

ECMA (µM)	DIV	hours after treatment: 2	23	47	72	95	120
12.5	9	45%	-	N.S	N.S	-	N.S
	12	30%	N.S	N.S	-	N.S	-
25.0	9	61%	-	N.S	23%	-	28%
	12	34%	28%	24%	-	26%	-
50.0	9	58%	-	54%	54%	-	43%
	12	54%	39%	29%	-	-	-

All values significant $P<0.05$; N.S : not significant, Values derived from 6 independent cultures each of 20 flasks sampled at different times and assayed independently

Using 12.5 µM ECMA, QNB binding was inhibited by up to 45% within 2h, showing full recovery to control values within 24h regardless of the age of the cultures at treatment (Table 1). 25 and 50 µM ECMA gave similar initial inhibition, but recovery was less marked and certainly not complete even after 120h. In age-matched control cultures, QNB binding increased linearly by approx 45-75% over at least 96h. Using membrane preparations from adult rats, inhibition of QNB binding was irreversible by washing (IC_{50},10 min reaction, approx 40 µM). Suspension of membranes in culture medium and reaction with ECMA (12.5 or 50 µM) for 2h gave irreversible inhibitions comparable with those obtained with cell cultures in 2h, with similar lack of dependence on the dose of ECMA.

The accessibility and sensitivity of muscarinic receptors to ECMA in cultures is clearly no different from those in subcellular membrane preparations. Both are extensively blocked in spite of the rapid clearance of ECMA from the culture medium (Pillar et al, 1987). Furthermore, recovery of QNB binding in cultures is not by dissociation of ECMA, suggesting resynthesis of receptors. If so recovery to control concentrations within 24-48h, after 12.5 µM ECMA, is at rates of synthesis greater than control. This implies 50 µM ECMA evokes a wider response, resulting in less marked net recovery, possibly by loss of cells additional to cholinergic neurones and possessing muscarinic receptors. 12.5 µM ECMA, a concentration comparable with those used in vivo, causes a final lesion of cholinergic neurones in these cultures analogous to those caused in vivo and found in Alzheimer's disease.

Atterwill et al (1984) Br J Pharmac 83 89-102. Atterwill et al (1986) Br J Pharmac 88 355P. Fisher & Hanin (1986) Ann Rev Pharmacol Toxicol 26 161-181. Leventer et al (1985) Neuropharmacol 24 453-459. Pillar et al (1987) Arch Toxicol, in press. Pillar et al (1987) Br Pharmac Soc, Oxford meeting.

INHIBITION BY LITHIUM OF HYDROLYSIS OF MYO-INOSITOL-1-PHOSPHATE AND MYO-INOSITOL-4-PHOSPHATE IN BOVINE BRAIN

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Recent reports have indicated that in liver inositol-1,4-bisphosphate is metabolised to free inositol either via a two step Li^+-insensitive pathway involving the formation of inositol-4-phosphate (I4P), or via a two step Li^+-sensitive pathway involving the production of inositol-1-phosphate (I1P) with both steps being equally sensitive to Li^+ (Storey et al, 1984; Michell et al, 1986). In brain, I1P is hydrolysed by a Li^+-sensitive myo-inositol-1-phosphatase (Hallcher and Sherman, 1980), an effect which may underlie the therapeutic response to Li^+ salts in the treatment of manic-depressive illness. Little is known, however, about the production or metabolism of I4P in CNS, although small increases in I4P levels have been measured in the brains of Li^+ treated rats (Sherman et al, 1985). In the present study, we report that myo-inositol-1-phosphatase (I1P'ase) from bovine brain hydrolyses both I1P and I4P. Moreover, both activities are inhibited by Li^+ ions.

A partially purified preparation of I1P'ase was obtained from whole bovine brain according to the method of Hallcher and Sherman (1980) with minor modifications. Hydrolysis of (±)-I1P was determined at 37°C in assay buffer containing 0.25M KCl, 50mM Tris-HCl, 3mM $MgCl_2$, (pH 7.8 at 20°C), either by measuring the production of inorganic phosphate (Itaya and Ui, 1966) or by determining the formation of [^{14}C]-inositol from [^{14}C](+)-I1P (Amersham; spec. act 55mCi/mmol) using Dowex anion exchange chromatography. Hydrolysis of (±)-I4P was examined under the same assay conditions using the above phosphate release assay. Both (±)-I1P and (±)-I4P were hydrolysed by the above enzyme preparation. Initial rate data were determined at several concentrations of each substrate and yielded mean Km values of 0.124 ± 0.007 mM (n=13) for (±)-I1P and 0.072 ± 0.01 mM (n=5) for (±)-I4P. To examine the effects of Li^+, enzyme was incubated with 0.1mM substrate for 10 min to allow 30-40% conversion of substrate to product. In agreement with Hallcher and Sherman (1980), Li^+ induced a dose-dependent inhibition of (±)-I1P hydrolysis, reducing enzyme activity to <5% at 30 mM. When incubated with (±)-I4P, Li^+ induced a similar dose-dependent inhibition. IC_{50} values for Li^+ versus (±)-I1P and (±)-I4P hydrolysis were 1.27 ± 0.07 mM (n=10) and 0.346 ± 0.028 mM (n=5), respectively. Although the above differential Li^+ sensitivity could suggest the existence of two separate inositol monophosphatases, the presence of (±)-I4P markedly reduced the conversion of [^{14}C](+)-I1P to [^{14}C]-inositol implying that both (±)-I4P and (±)-I1P are hydrolysed by the same inositol monophosphatase. This has been confirmed by purification of I1P'ase to homogeneity. The homogenous enzyme catalyses the hydrolysis of (±)-I1P and (±)-I4P at relative rates similar to those observed in either crude brain homogenates or the above partially purified enzyme, and both activities are inhibited by Li^+. In conclusion, our results support the existence of a single inositol monophosphatase in bovine brain, capable of hydrolysing both (±)-I1P and (±)-I4P in a Li^+-sensitive manner.

Hallcher L. and Sherman W. (1980) J. Biol. Chem. 255, 10896-10901.
Itaya K. and Ui M. (1966) Clinica Chimica Acta 14, 361-366.
Michell R., Cubitt A., Downes C., Hawkins P., King C., Kirk C., Morris A., Mussat M., Shears S. and Storey D. (1986) Hormones and Cell Regulation, Vol. 139 (eds. Dumont J. & Nunez J.) p.71-79.
Sherman W., Munsell L., Gish B. and Honchar M. (1985) J. Neurochem 44, 798-807.
Storey D., Shears S., Kirk C. and Michell R. (1984) Nature 312, 374-376.

GABA INHIBITS HISTAMINE-INDUCED INOSITOL PHOSPHOLIPID BREAKDOWN IN GUINEA-PIG CEREBELLUM

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Histamine H_1-receptors are distributed widely in guinea-pig brain, although there is little indication of their functional role. There is an increasing interest in histamine as a neuromodulator, but the only interaction that has been studied in any detail is that between histamine and adenosine (Hollingsworth & Daly, 1985; Hill & Kendall, 1986). In guinea-pig cerebellum, the region of guinea-pig brain with the highest density of [^{3}H]-mepyramine binding sites, the H_1-receptors appear to be associated with the dendrites of Purkinje cells (Palacios et al, 1981) and local application of histamine leads to an inhibition of cell firing (Mahawala et al, 1981). In the rat the excitability of Purkinje cell dendrites is depressed by GABA (Malenka & Kocsis, 1982), an observation which suggests the possibility of an interaction between histamine and GABA. To test for such an interaction in guinea-pig cerebellum we have examined the effect of GABA on histamine-induced inositol phospholipid breakdown, the only biochemical response to H_1-receptor activation reported in this tissue (Daum et al, 1983).

Measurement of [^{3}H]-inositol 1-phosphate ([^{3}H]-IP_1) formation in cross-chopped slices (350 x 350 um) from guinea-pig (Dunkin-Hartley strain, males) cerebellum was carried out essentially as described previously (Carswell & Young, 1986). The slices were labelled with 0.3 uM [^{3}H]-inositol for 30 min and incubation with histamine and/or GABA-mimetics in the presence of 10 mM LiCl was terminated by addition of trichloroacetic acid.

GABA produced a dose-dependent inhibition of both basal and histamine-stimulated accumulation of [^{3}H]-IP_1. After a 30 min incubation with 2 mM GABA, which gave a near maximal effect, or 2 mM GABA + 0.2 mM histamine the inhibition was 24 $\pm$ 3 and 45 $\pm$ 6% (n=3), respectively, while after 60 min incubation the corresponding values were 30 $\pm$ 2 and 46 $\pm$ 2% (n=6). The GABA uptake inhibitor nipecotic acid (1 mM) also inhibited both basal and histamine-stimulated [^{3}H]-IP_1 accumulation (29 and 38%, respectively, after 30 min incubation and 48 and 47% after 60 min). However, (-)-baclofen (100 uM and 1 mM) and muscimol (100 uM and 1 mM) had no significant effect and the inhibition produced by 2 mM GABA was not reduced by 100 uM bicuculine methobromide. These observations indicate that there is an interaction between GABA and histamine in guinea-pig cerebellum, but the effect of GABA does not seem to be exerted through receptors with either a $GABA_A$ or $GABA_B$ character. The basal accumulation of [^{3}H]-IP_1 in guinea-pig cerebellum is high and the inhibitory action of GABA suggests that it might be due to the release of an endogenous mediator, although phentolamine (1 uM), methylatropine (1 uM), TTX (1 uM) and ETYA (15 uM) were all without effect. The effect of nipecotic acid on the basal level may indicate the formation of significant amounts of GABA by the slice preparation.

Carswell, H. & Young, J.M. (1986) Br. J. Pharmac. 89, 809-817
Daum, P.R. et al (1983) Eur. J. Pharmac. 87, 497-498
Hill, S.J. & Kendall, D.A. (1986) Br. J. Pharmac. 89, 771P
Hollingsworth, E.B. & Daly, J.W. (1985) Biochim. Biophys. Acta 847, 207-216
Malenka, R.C. & Kocsis, J.D. (1982) J. Neurophysiol. 48, 608-621
Marwaha, J. et al (1981) Exp. Neurol. 74, 285-292
Palacios, J.M. et al (1981) Brain Res. 214, 155-162

POSSIBLE COEXISTENCE OF CARRIERS FOR GABA AND CHOLINE UPTAKE ON THE SAME NERVE TERMINAL IN RAT HIPPOCAMPUS

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Modulation of neurotransmitter release at nerve endings occurs, in general, through receptors located on the releasing terminals. Here we show that GABA increased the release of [^{3}H]ACh from rat hippocampus nerve terminals prelabelled with [^{3}H]choline ([^{3}H]Ch) without apparent involvement of GABA receptors. The GABA effect was prevented by inhibitors of GABA uptake. Conversely, the release of endogenous GABA was enhanced by Ch and the effect was counteracted by the Ch uptake inhibitor hemicholinium-3. Thus it appears that some cholinergic terminals in rat hippocampus possess a carrier for GABA uptake whereas some GABAergic nerve endings possess a Ch transporter.

Rat hippocampal synaptosomes were prelabelled with [^{3}H]Ch and exposed to GABA. The basal release of [^{3}H]ACh was increased (25-65%) by GABA (0.01-0.3 mM). Bicuculline or picrotoxin did not antagonize the effect of the aminoacid. Muscimol (0.3 mM) had a negligible effect on the release of [^{3}H]ACh. (-)Baclofen, up to 0.3 mM, did not increase significantly [^{3}H]ACh efflux. The findings tend to exclude an involvement of $GABA_A$ or $GABA_B$ receptors. The enhancement of [^{3}H]ACh release elicited by GABA was counteracted by three compounds, SK&F 89976A, SK&F 100561 and SK&F 100330A, which are potent and selective inhibitors of neuronal GABA uptake (Yunger et al., 1984). At a concentration of 0.01 mM these drugs prevented almost totally the effect of 0.1 mM GABA. One likely interpretation for this finding is that GABA stimulates ACh release because it is taken up by a selective GABA carrier located on nerve terminals which also possess a Ch transport system and can synthesize ACh.

The observation that ACh was released when GABA was taken up prompted us to investigate whether, reciprocally, the release of GABA could be affected during Ch uptake. Choline (0.01-0.3 mM) caused a concentration-dependent stimulation (25-70%) of the basal release of endogenous GABA from hippocampus synaptosomes. Moreover, the releasing effect of Ch was counteracted by hemicholinium-3. Carbachol, ACh or oxotremorine, at 0.1 mM, did not affect the release of endogenous GABA. The effect of Ch was not prevented by atropine. It could be concluded that Ch elicited release of GABA when it was taken up by an hemicholinium-sensitive Ch carrier located on the GABA-releasing terminals.

Taken together the results raise the possibility that transmitter release is modulated by a mechanism involving penetration of the modulators through selective carriers located on the releasing terminals. Moreover, the coexistence of Ch and GABA carriers on the same nerve ending might be compatible with the idea that ACh and GABA coexist in some terminals of rat hippocampus and reciprocally affect release.

Supported by Italian C.N.R. and Ministry of Education

Yunger, L.M. et al., (1984) J. Pharmacol. Exp. Ther. 228, 109-115.

[^{3}H]-SULPIRIDE AUTORADIOGRAPHY IN CONTROL RAT AND MONKEY BRAINS AND IN THOSE LESIONED WITH 6-OHDA AND MPTP RESPECTIVELY

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Sulpiride is a selective dopamine (DA) D2 receptor ligand. This report describes the distribution of 3H-sulpiride binding in striatal areas of the rat and monkey, and how it is affected by loss of dopaminergic input to these areas using 6-hydroxydopamine (6OHDA) treatment in the rat and 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) treatment in the monkey.

Unilateral 6OHDA lesions were performed stereotaxically in male Sprague Dawley rats. Those showing marked contralateral turning after apomorphine (0.5 mg/kg s.c. at least 5 days post-lesion) were used in the autoradiographic studies. Three monkeys (Macaca fascicularis) were treated with MPTP (0.3-0.7 mg/kg i.v. over 7-8 days). All became hypokinetic, rigid and exhibited varying degrees of tremor. Rats were killed by decapitation 30 days post-lesion and monkeys by pentobarbitone overdose 21 days after the first dose of MPTP. Brains were rapidly frozen in isopentane and stored at -70°C. Cresyl violet staining revealed a loss of pars compacta cells of the substantia nigra in both groups of animals, together with a 90% depletion of striatal DA levels in the monkey. Cryostat-cut brain sections (20um) were thaw-mounted on gelatin-coated glass slides. Sections were preincubated at room temperature for 15 min in 50mM TRIS HCl pH 7.4. Incubations, in the same medium, with 120mM NaCl and 15-20nM 3H-sulpiride, were at 37°C for 20 min. Specific binding was defined as that displaced by 1uM sulpiride. Sections were washed in ice-cold buffer, dipped in distilled water, air-dried and exposed to LKB film for 28 days at -20°C.

Both control rat and monkey brains showed a heterogeneous pattern of 3H-sulpiride binding in the striatum, with an increasing medial to lateral gradient. This is in agreement with Joyce et al. (1985) who have shown similar results with 3H-spiroperidol in the rat, and Kohler and Radesater (1986) who have revealed D2 sites in the monkey using 3H-raclopride. Highest levels of binding varied along the rostro-caudal axis of the striatum in the rat and the putamen in the monkey. The greatest binding at rostral levels was found dorsolaterally, whereas it occurred ventrolaterally at more caudal levels. The dorsolateral quadrant of the rostral rat striatum has been shown to contain "hyperdense" patches of DA axon terminals (Doucet et al., 1986). Treatment with 6OHDA and MPTP caused an increase in specific 3H-sulpiride binding compared with contols. In the denervated striatum of the rat there was a 70% increase, most evident rostrally. In the monkey there was a 50-100% increase in the mid and caudal regions of the caudate nucleus and the putamen.

The increase in D2 receptor number revealed in this study, particularly in the primates, where parkinsonian symptoms were present, could be indicative of the underlying changes in these receptors in untreated Parkinson's disease in man.

Doucet, G. et al (1986) Neuroscience 19, 427
Joyce, J.N. et al (1985) Brain Research 338, 209
Kohler, C. & Radesater, A-C. (1986) Neuroscience Letters 66, 85

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Receptors for the endogenous excitatory acids, glutamic and aspartic acids, can be divided into three classes according to their selectivity for three exogenous amino acids. These are the NMDA-preferring, the quisqualate-preferring and the kainate-preferring subtypes. Recently, Drejer et al. (1986) have reported that the receptor subtype controlling aspartate release from cultured cerebellar granule cells cannot be accommodated in this classification. We now have confirmed and extended the pharmacological characterisation of this site.

Cultures were prepared from neonatal rat cerebellum as described by Graham et al. (1976). These cultures contained clusters of granule cells essentially devoid of glial elements. The cells were incubated with [^{3}H]D-aspartic acid (180 nM/, 30 min, 37°C; 1 µCi/dish) which was uptaken into the cells. The cells were then perfused with Krebs-HCO_3 buffer into which pulses (2 min) of depolarising stimuli could be introduced. The [^{3}H]D-aspartate was released into the medium by potassium depolarisation or application of EAAs. Release of aspartate could be stimulated by glutamic and ibotenic acids (a tripling of release at a concentration of 10^{-5} M for both amino acids), but not by NMDA or kainic acid; the action of ibotenic acid thus does not seem to be due to an interaction with a NMDA-preferring receptor. Quisqualic acid ($>10^{-4}$ M) did release aspartate, but not at concentrations appropriate to the activation of its own subtype of EAA receptor.

The stimulatory effects of glutamic and ibotenic acids on aspartate release could be antagonised by O-phosphoserine with an IC_{50} of 4×10^{-5} M. The antagonist activity of this compound resided entirely in the l-isomer. Other antagonists in this model were α-aminoadipic acid ($IC_{50} = 5 \times 10^{-4}$ M) and PK 26124 ($IC_{50} = 1 \times 10^{-5}$ M), (Benavides et al. 1985) Weak antagonist activity was seen with high doses of phencyclidine (33 % inhibition at 10^{-5} M), 2 APV (52 % inhibition at 10^{-3} M) and 2 APB (30 % inhibition at 10^{-3} M).

The pharmacology of the EAA receptor controlling EAA release from the cerebellar granule cell does not conform to that of any of the three subtypes of EAA receptor defined electrophysiologically nor to the Ca^{++}/Cl^- dependent [^{3}H]-glutamic acid binding site sensitive to 2-APB. It is, however, similar to the EAA receptor coupled to phosphatidylinositol turnover in neonatal rat hippocampus described by Nicoletti et al. (1986). There might thus be a fourth, ibotenate-preferring, subtype of EAA receptor at which O-phospho-L-serine would be a selective antagonist.

Drejer et al. (1986) Life Sci., 38, 2077.
Graham et al. (1976) Brain Res. 115, 181.
Benavides et al. (1985) Neuropharmacology 24, 1085.
Nicoletti et al. (1986) Neuropharmacology 25, 2077.

THE EFFECTS OF 5-HYDROXYTRYPTAMINE AND SELECTIVE 5-HT BINDING LIGANDS ON THE EXCITABILITY OF RAT SPINAL MOTONEURONES

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5-Hydroxytryptamine (5HT) binding in the central nervous system and the behavioural effects of selective binding ligands have formed the basis of a tentative classification of 5HT receptor types (Bradley et al. 1986). Davies et al. (1985a,b;1986) have studied the effects of selective ligands applied by microiontophoresis to midline brainstem neurones and concluded that cell excitation is mediated by a 5HT 2 receptor which is blocked by ketanserin. Depression of cell activity is mediated by a 5HT 1-like receptor which is not blocked by ketanserin and the agonists, 8 OH DPAT and 5-Carboxamidotryptamine (5CT), are more potently depressant than 5HT.

We have extended our studies to include the effects of 5HT on motoneurones by studying the population spike which is antidromically evoked by ventral root stimulation (Barasi and Roberts, 1974; Lipski, 1981). Stimulation of nucleus raphe obscurus and iontophoretic applications of 5HT both strongly facilitate this response. This facilitation was not blocked by ketanserin applied iontophoretically (40 nA for 20 min) or intravenously (2 mg/kg). As ketanserin is a very selective and potent 5HT 2 antagonist it is unlikely that this effect of 5HT is mediated by 5HT 2 receptors. The poorly selective 5HT antagonist methysergide (1-2 mg/kg) was effective, however, in reducing the 5HT response without affecting the response to noradrenaline. The effects of 5HT were potently mimicked by 5CT which suggests that a 5HT 1-like receptor was involved. However, 8 OH DPAT was not an effective agonist (100 nA for 1 min) and cyanopindolol did not block the 5HT response with applications which abolished the response to noradrenaline (25 nA for 10-20 min).

To determine if the effects of the agonists were mediated by presynaptic receptors, the experiments were repeated in rats which had received intracerebroventricular 5,7 DHT two weeks previously. These rats had a 95% reduction in spinal 5HT concentrations and very few immunofluorescent 5HT fibres around motoneurones. The responses to 5HT were strongly potentiated in these animals although responses to raphe obscurus stimulation were almost abolished.

In conclusion it appears that postsynaptic 5HT 1-like receptors which are not affected by 8 OH DPAT or cyanopindolol are responsible for an increase in motoneurone excitability. These excitatory receptors differ from excitatory receptors in the brainstem which are probably 5HT 2 and also differ from the depressant 5HT 1 receptors on brainstem neurones where 8 OH DPAT is an effective agonist.

Bradley,P.B. et al. Neuropharmacology (1986) 25 563-576.
Barasi,S. and Roberts,M.H.T. (1974) Br. J. Pharmac. 52 339-348
Davies,M. et al. (1985a) Br. J. Pharmac. 85 255P
Davies,M. et al. (1985b) Br. J. Pharmac. 86 594P
Davies,M. et al. (1986) Br. J. Pharmac. 89 526P
Lipski,J. (1981) J.Neurosci. meth. 4 1-32

AN IN VITRO SPINAL CORD PREPARATION FROM THE MATURE RAT

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In vitro preparations from amphibia (Curtis, Phillis & Watkins 1961) or immature rats (Konishi & Otsuka 1974) have provided robust preparations convenient for the measurement of drug effects on spinal neurones. We report in this paper that a stable in vitro preparation, with characteristics similar to the widely used immature preparation, can be prepared from the sacral and coccygeal segments of the mature (180 - 250g body weight) rat.

Spinal cords were removed from urethane (2g per Kg body weight i.p.) anaesthetized animals and placed immediately in Ringer solution containing 100mM urethane. The spinal cords were hemisected in the urethane containing Ringer solution then placed in the recording apparatus and superfused with urethane-free Ringer solution (composition NaCl 111mM, KCl 3mM, $NaHCO_3$ 24mM, $CaCl_2$ 2.5mM, $MgSO_4$ 1mM, glucose 12mM. Gassed with 95% O_2 5% CO_2 at 25°C as described for the immature preparation (Evans and Watkins 1978).

After washout of urethane supramaximal electrical stimulation of dorsal roots (S2 - Co1) evoked polyphasic reflexes (DR-VRP) in corresponding and adjacent ventral roots. The earliest component of such responses consisted of a compound action potential (CAP) superimposed on a population excitatory postsynaptic potential (epsp) of motoneurones recorded in corresponding ventral roots. Latency to first positive going deflection (reflecting depolarization of motoneurones) 1.1 msec ± 0.1 s.e. mean. Peak amplitude of CAP 9.0 mV ± 1.4 s.e. mean (9 preparations). Time to half decay of epsp 12.5 msec ± 0.6 s.e. mean. A late component (peak amplitude 0.33 mV ± 0.07 s.e. mean, latency to peak 76.7 msec ± 5.2 s.e. mean, time to half decay 125 msec ± 28 s.e. mean) of the DR-VRP was present in 3 out of the 5 preparations measured over this time course. In the two preparations, in which it was not detected initially, removal of Mg^{2+} from the bathing medium resulted in the appearance of the late component (peak amplitude 1.2 mV ± 0.4 s.e. mean, latency to peak 70 msec ± 13 s.e. mean, time to half decay > 300 msec, 3 preparations). These responses were maximally evoked by 0.1 to 1 msec pulses at intensities between 2 and 10 times threshold.

Kynurenic acid (1mM) produced marked depression of all components of DR-VRPs (CAP depressed to 32.4% control ± 12.7 s.e. mean, n = 3). The N-methyl-aspartate antagonist (±)2-amino-5-phosphonopentanoate (AP5) (50μM) had only a small effect on the initial part of the DR-VRP (90.7% control ± 2.1 s.e. mean, n = 3) but abolished the late component in the absence or presence of 1 mM Mg^{2+}.

In the absence of Mg^{2+} preparations showed marked AP5-sensitive spontaneous synaptic activity which was depressed by glycine, gamma-aminobutanoate (2-5 mM) or taurine (1-2 mM) (2 preparations). The depressant effects of glycine and taurine, but not GABA, were reversed by strychnine (1 μM). Similar APV-sensitive spontaneous activity was induced in the presence of Mg^{2+} by strychnine (1 μM, 2 preparations).

This preparation will be useful for the assessment of drug action on reflexes and motoneurones of the mature mammalian spinal cord.

Curtis, D.R., Phillis, J.W. & Watkins, J.C. (1961) Br. J. Pharmac. 16, 262-283.
Evans, R.H. & Watkins, J.C. (1978) Eur. J. Pharmac. 50, 123-129.
Otsuka, M. & Konishi, S. (1974) Nature 252, 733-734.

ACTIONS OF MPTP AND MPP^+ ON GUINEA-PIG HIPPOCAMPAL NEURONES IN VITRO

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MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) induces via the metabolite MPP^+ (1-methyl-4-phenylpyridinium) a Parkinson's disease-like syndrome in man and many mammals (Langston, 1985). In addition to this chronic effect on dopaminergic neurones, a variety of acute effects of MPTP have been observed in monkeys, cats and guinea-pigs, including salivation, diarrhea, dilated pupils, convulsions and death (Chieuh et al, 1984; Schneider et al, 1986). These symptoms probably arise from actions of MPTP on structures outside the nigrostriatal system and so we studied the effects of MPTP and MPP^+ on synaptic transmission and single neurone properties in the hippocampal slice preparation. Recordings were made intra- and extracellularly in the CA1 region and the Schaffer collateral pathway was electrically stimulated.

Addition of 50-100µM MPTP to the superfusate produced the following effects. (a) The amplitudes of the excitatory postsynaptic potentials and extracellularly recorded population spikes transiently (ca. 10-20min) increased and then decreased to zero; synaptic transmission was completely blocked after about 40min. This was reversible after several hours washing in drug-free solution. (b) The block, but not the facilitation of synaptic transmission was prevented by prior incubation in 10µM pargyline, a monoamine oxidase inhibitor, which prevents the metabolism of MPTP to MPP^+. (c) Intracellular recordings from single pyramidal neurones revealed that MPTP (100µM) had no significant effect on resting membrane potential or resistance. Action potentials elicited by depolarizing current pulses were also unaffected. (d) MPP^+ (50µM) and the proposed metabolic intermediate $MPDP^+$ (1-methyl-4-phenyl-2,3-dihydro-pyridinium; 50µM) both blocked synaptic transmission by a pargyline-insensitive mechanism. (e) Axonal action potentials, recorded in stratum radiatum, were slightly (ca. 10%) increased in amplitude by MPTP and MPP^+.

The results of this investigation show that MPTP, MPP^+ and $MPDP^+$ have significant acute effects on neurones in the hippocampus. Thus the actions of these toxins are not restricted to dopaminergic or nigrostriatal systems. The block of synaptic transmission most probably results from an action of MPP^+ at a presynaptic site.

This research was supported by SFB 220, project B2.

Chieuh, C.C. et al (1984) Psychopharmacol. Bull. 20,548-553
Langston, J.W. (1985) Trends in Neurosci. 8,79-83
Schneider, J.S. et al (1986) Exp. Neurol. 91,293-307

SYSTEMIC ADMINISTRATION OF MK-801 PROTECTS AGAINST ISCHAEMIC NEUROPATHOLOGY IN RATS

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MK-801 is a potent, selective and non-competitive antagonist of the N-methyl-D-aspartate (NMDA) sub-type of excitatory amino acid receptors (Wong et al, 1986), which protects (ED_{50} = 0.3mg/kg) against ischaemia-induced hippocampal neurodegeneration in the gerbil, when given systemically either pre or post-ischaemically (Foster et al, 1986). This supports the idea that NMDA receptors are fundamentally involved in the neuropathology of ischaemia-induced neuronal damage. In a further investigation of the neuroprotective action of MK-801 we evaluated its effect in acute and recovery models of rat cerebral ischaemia.

The acute ischaemia model used was as described by Simon et al (1984), MK-801 (10mg/kg i.p.) was administered 1h prior to a 30 min ischaemic insult. Following 2h of reperfusion animals were perfusion fixed. Cresyl violet stained coronal sections (40mm) were assessed blind for ischaemic cell changes in the hippocampus and the percentage of neurones affected was assessed in pyramidal areas CA1, CA2, CA3 and CA4 for each animal. The recovery model used was essentially as described by Smith et al (1984), with the exceptions that a neuromuscular blocker was not used and the anaesthetic mixture was 1% isoflurane, 30% O_2, 70% N_2O, MK-801 (0.3mg/kg i.p.) was administered 1h prior to a 10 min period of cerebral ischaemia. Rats were perfusion fixed 7 days following the ischaemia and neuronal damage was assessed blind by counting the number of normal appearing pyramidal neurones in a 400mm length of the CA1 pyramidal cell layer from each hippocampus in cresyl violet stained coronal sections (20mm). The number of cells from both hippocampi were summed giving the total number of cells in a length of 800mm the mean ± SEM was calculated for the two groups (n = 7).

In acute ischaemia, pyknotic pyramidal neurones were observed in the hippocampi of control animals. Pretreatment with MK-801 significantly reduced the percentage of pyknotic neurones in all hippocampal areas.

Table 1: Effect of MK-801 (10mg/kg) in acute rat ischaemia

	% of ischaemic cells in hippocampal areas (Mean ± SEM)			
	CA1	CA2	CA3	CA4
Control (n = 5)	40 ± 15.8	72 ± 16.7	92 ± 5.5	76 ± 4.5
MK-801 (n = 5)	0*	4 ± 4.5*	20 ± 12.3**	28 ± 5.5**

*P < 0.05; ** P < 0.01 (unpaired t-test).

In the recovery model, control animals showed degeneration of hippocampal CA1/CA2 area pyramidal neurones (number of surviving neurones = 40.6 ± 9.27). Pretreatment with 0.3mg/kg of MK-801 (number of surviving neurones = 185 ± 29.47) significantly ($P \leq 0.002$, unpaired t-test) protected the animals against ischaemic neurodegeneration. These results show that MK-801 administered systemically protects against ischaemia-induced neuropathology in the acute and recovery models of rat ischaemia, further substantiating the hypothesis that NMDA receptors mediate neuronal degeneration caused by cerebral ischaemia.

Foster A.C., Gill R., Iversen L.L. and Woodruff G.N. (1986) Br. J. Pharmac. Proc. Suppl. 90:9P.
Wong E.H.F., Kemp J.A., Priestley T., Knight A.R., Woodruff G.N. and Iversen L.L. (1980) Proc. Natl. Acad. Sci. 83: 7104-7108.
Simon R.P., Swan J.H., Griffiths T and Meldrum B.S. (1984) Science, 226: 850.
Smith M.L., Bendek G., Dahlgren N., Rosen I., Wieloch T. and Siesjo B.K. (1984) Acta. Neurol. Scand. 69: 385-401.

THE DELAYED POST-ISCHAEMIC TREATMENT EFFECTS OF NICARDIPINE IN A RAT MODEL OF FOUR VESSEL OCCLUSION

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Hypotheses invoking an unregulated extension of the usual intracellular properties of calcium as a principal cytotoxic factor leading to central neuronal death in ischaemia are now well known (Hass, 1981, Siesjo, 1981). The neurocytoprotective effect of nicardipine given prophylactically (500µg.kg^{-1} i.p. 15 min pre-ischaemia), followed by post ischaemia maintenance treatment (500µg.kg^{-1} i.p. twice daily during 72 h survival) in a rat model of four vessel occlusion has been reported (Alps & Hass, 1985). Valid preparations, with blood pressure controlled by halothane-nitrous oxide in oxygen anaesthesia during a 10 min period of brain ischaemia were those showing at least 8 min of EEG isoelectricity. In the present studies the above treatment regimen with nicardipine has been delayed entirely into the post-ischaemia reperfusion period in different groups of rats (15min, n=5; 1h, n=6; 16-22h, n=5).

Selected brain slices were embedded in paraffin wax, sectioned at 7µM and stained with cresyl fast violet. Ischaemic cell change (ICC) was scored blind for central neurones using a subjective rating system from 0-4, where 0=normal appearance, 1=0-10%, 2=10-25%, 3=25-50%, and 4=>50% ICC. The number of values determined for each brain depended upon the size of the structure concerned (hippocampal CA_1 subfield = 2, hippocampal and striatal cortex each n=4, striatum =2, cerebellar Purkinje cells = 1). The scores were totalled to give group mean brain values. Drug treatment effects were compared against the value (2.07 $\pm$ 0.10) obtained for saline treated controls (n=11). A group of normal rats (n=6) were included to observe artifactual changes (0.43 $\pm$ 0.06).

Delayed treatment at 15 min post-ischaemia significantly protected all brain areas (ICC score 0.66 $\pm$ 0.10 $P<0.001$)> At 1h the CA_1 neurones were less protected but the mean score was low (0.60 $\pm$ 0.11, $P<0.001$). Although both the CA_1 neurones and Purkinje cells were unprotected in the 16-22h group the mean score was lower (1.19 $\pm$ 0.12, $P<0.001$) than the control value. Hippocampal CA_1 neurones and Purkinje cells are known to be more highly sensitive to ischaemia than other neurones. Provided nicardipine treatment is given prophylactically or commenced promptly it would appear to afford a high degree of protection to these neurones. Less susceptible neurones in other areas can be protected even if treatment is considerably delayed.

Alps, B.J. & Hass, W.K. (1985). Neurology, 35 (suppl 1), 141.
Hass, W.K. (1981) In: Cerebral Vascular Disease (eds Meyer, J.S., Lechner, H., Reivich, M., Ott, E.O., Aranibar, A.) pp 3-17, Exerpta Medica, Amsterdam.
Siesjo, B.K. (1981) J. Cereb. Blood Flow Metab., 1, 155-185.

CEREBRAL ISCHAEMIA REDUCES ^{5}-HT$_2$ BINDING SITE DENSITY BUT NOT AFFINITY

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One of the consequences of cerebral ischaemia in the mongolian gerbil, meriones unguiculatus, is a depletion of cerebrocortical 5-hydroxytryptamine (5HT) (Welch et al, 1978; Alps et al, 1985). We have now investigated whether cerebral ischaemia as a result of unilateral carotid artery ligation also induces changes in the affinity (Kd) or density (Bmax) of 5HT$_2$ binding sites in the frontal cortex of the gerbil.

Cerebral ischaemia was induced for 3 h in the right hemisphere of anaesthetised male gerbils (60-80 g) as described by Alps et al 1985. Animals with an intact circulus arteriosus , which are resistant to unilateral ischaemia, were discarded. The frontal cortex from stroke-prone animals (30%), i.e. with an incomplete circulus arteriosus, was separated into left and right hemispheres.

Frontal cortices, from 2 pooled left or right hemispheres, were homogenised in 50 mM Tris HCL, pH 7.4, and centrifuged at 38,000gav for 10 min. After two further washes, the homogenates were incubated for 15 min at 37^{o}C and centrifuged again. The final pellet was resuspended in 50 mM Tris HCL, pH 7.7, at 25^{o}C, containing 5.7 mM ascorbate, 10 µM pargyline and 4 mM $CaCl_2$, incubated for 15 min at 37^{o}C and cooled on ice before use in the binding assay. In saturation experiments membranes were incubated in a final volume of 1 ml for 15 min at 37^{o}C with concentrations of [^{3}H]-ketanserin ranging from 0.05-5 nM. Bound ligand was separated from free by vacuum filtration. Competition experiments were performed using a fixed concentration of [^{3}H]-ketanserin (1 nM). Non-specific binding was determined in the presence of 2 µM methysergide.

The binding of [^{3}H]-ketanserin to gerbil frontal cortex was saturable and characteristic of the 5HT$_2$ binding site, having a high affinity for spiperone (Ki 0.8nM), cyproheptadine (Ki 5.2nM), methysergide (Ki 15nM) and low affinity for 5HT (Ki 1050nM). Scatchard transformations of the equilibrium binding isotherms indicated that ketanserin bound to a single class of high affinity binding sites with a Kd of 0.48 nM and a Bmax of 206 fmol/mg protein. Unilateral ligation caused a significant decrease ($p < 0.001$) in the number of 5HT$_2$ binding sites in the right but not the left hemisphere of the gerbil frontal cortex without an apparent change in affinity (Table 1).

Table 1. Effect of ischaemia on 5HT$_2$ binding parameters in gerbil frontal cortex

	Left hemisphere		Right hemisphere	
	Kd (nM)	Bmax (fmol/mg protein)	Kd (nM)	Bmax (fmol/mg protein)
Control	0.48 ± 0.03	203.4 ± 10.4	0.47 ± 0.03	209.4 ± 18.9
Ligated	0.51 ± 0.03	218.4 ± 7.0	0.45 ± 0.02	* 95.5 ± 10.0

* $p < 0.001$ relative to control animals; mean ± s.e.mean, n=5 (10 animals/group)

It remains to be seen whether the marked reduction in the number of 5HT$_2$ sites is merely a consequence of cerebral ischaemia or one of the factors exacerbating ischaemic damage.

Alps, B.J., et al. (1985) Biochem. Soc. Trans., 13(2), 384.
Welch, K.M.A., et al. (1978) Ann. Neurol., 3, 152-155.

CHARACTERISATION OF THE ANTAGONISM OF EXCITATORY AMINO ACID RECEPTORS IN RAT CORTEX BY KYNURENIC ACID

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Kynurenic acid (KYNA) is a "broad spectrum" excitatory amino acid antagonist which blocks responses mediated by the N-methyl-D-aspartate (NMDA), quisqualate and kainate receptor subtypes, and has been widely used to identify excitatory amino acid-using synapses in the mammalian central nervous system. As part of our pharmacological characterisation of excitatory amino acid receptors (Foster et al, 1986) we have examined the potency of KYNA, and its analog, 3-hydroxy-2-quinoxalinecarboxylic acid (HQC), on the three receptor subtypes in rat cortex using electrophysiological and radioligand binding techniques.

Electrophysiological experiments on rat cortical slices were performed as previously described (Kemp et al, 1986). Radioligand binding assays using crude post-synaptic densities (PSDs) from rat cortex were carried out in 50mM Tris-acetate buffer (pH 7.0), using L-[3H]-glutamate (50nM) for NMDA receptors, [3H]-2-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA, 36nM) for quisqualate receptors, and [3H]-kainate (16nM) for kainate receptors. Under the assay conditions employed, approx. 80% of specific L-[3H]glutamate binding to crude PSDs is displaceable by NMDA receptor-selective ligands. KYNA and HQC were able to inhibit 100% of L-[3H]glutamate binding with Ki's of 184 (176,193) (mean (-SEM, +SEM)) and 113 (78,164) μM and Hill coefficients of 0.86 ± 0.06 and 0.93 ± 0.23, respectively (N = 3 separate determinations). [3H]-AMPA binding was completely displaced with similar potencies; IC_{50}s = 101 (93, 110) and 139 (82, 233) for KYNA and HQC respectively (n = 3) with Hill coefficients = 1. In contrast, both these compounds were much weaker at displacing [3H]-kainate binding, with IC_{50}s of 2082 (1778, 2437) and 2185 (1991, 2398) μM for KYNA and HQC , respectively (n = 3). In the cortical slice experiments KYNA and HQC had similar pA_2 values against all the agonists tested (table 1). However, against NMDA receptor agonists (NMDA and trans-2,3-piperidine dicarboxylic acid (trans-2,3-PDA)) the slopes of the Schild plots were very steep, whilst for agonists of the other receptor subtypes they remained close to 1.

Table 1

	KYNA		HQC	
Agonist	pA_2	Slope (± 95% C.L.)	pA_2	Slope
NMDA	3.88	1.81 ± 0.14	3.77	2.06 ± 0.15
Quisqualate	3.92	0.86 ± 0.14	3.59	1.08 ± 0.26
Kainate	4.05	0.79 ± 0.13	3.80	0.97 ± 0.13
AMPA	3.86	1.08 ± 0.11	NT	NT
Trans-2,3,-PDA	3.81	1.93 ± 0.15	NT	NT

The steep slopes of the Schild plots for NMDA receptor agonists suggests that KYNA and HQC do not act in a simple competitive manner at this receptor subtype. This was also apparent in combination studies with KYNA and the competitive NMDA antagonist D-2-amino-5-phosphonovalerate. The fact that these compounds were effective against kainate-induced depolarisations but failed to show similar potencies in the binding experiments indicates that these responses may be mediated by a site other than that labelled by [3H]-kainate.

Foster, A.C., Grimwood, S. and Kemp, J.A. (1986) Br. J. Pharmac. 89, 869P.
Kemp, J.A., Priestley, T. and Woodruff, G.N. (1986) Br. J. Pharmac. 89, 535P.

THE EFFECTS OF REPEATED EXPOSURE TO HYDROGEN SULPHIDE ON AMINO ACID CONTENT OF THE BRAINSTEM

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Exposure to hydrogen sulphide (H_2S) is surprisingly common in industry, agriculture, and petroleum and natural gas exploration. Numerous fatalities and sub-lethal toxicities have occurred.

The lethality of H_2S has been attributed to its toxic effect on the respiratory centers (Beauchamp et al., 1984). Little or no information, however, exists on the effects of chronic administration of sub-lethal amounts of H_2S on neurochemical parameters. In this study two paradigms of chronic toxicity were adopted: 1) Ten male ICR mice (20-30 g) were treated once daily for 5 days with a dose of 5 mg/kg i.p. of NaHS (50% of LD_{50}) and 2) Six male Sprague-Dawley rats (300-350 g) were treated 3 times daily for 3 days with 7.5 mg/kg i.p. of NaHS (50% of LD_{50}). Control animals (n=12 and 8, respectively) received only saline. Animals were sacrificed 10 h following the last treatment and the brainstems frozen in isopentane cooled by liquid nitrogen. Tissues were homogenized in 0.1 N HCl and enzymes inactivated by heating at 100°C for 5 min. Homogenates were then centrifuged for 15 min to remove cell debris. An aliquot of each supernatant was derivatized with o-pthalaldialdehyde and then analyzed for aminoacid content using HPLC with fluorescence detection (Lindroth and Mopper, 1979). No changes in amino acid content of brainstems from the mice were evident. Among the rat brainstems glutamate levels declined as did the levels of glutamine and taurine. These data are summarized below in μmoles/g wet weight ± S.E.$\bar{x}$.

Table 1 Amino acid concentrations in brainstem

	MICE		RATS	
	CONTROLS (12)	TREATED (10)	CONTROLS (8)	TREATED (6)
Aspartate	2.32±0.25	2.58±0.25	2.71±0.36	1.89±0.29
Glutamate	5.15±0.53	5.16±0.42	5.90±0.39	3.42±0.42**
Glutamine	1.03±0.19	1.03±0.14	1.13±0.10	0.61±0.10*
Glycine	2.73±0.33	3.59±0.89	2.79±0.51	2.18±0.42
Taurine	4.08±0.62	3.93±0.70	2.19±0.41	1.09±0.20*
GABA	1.66±0.19	1.52±0.18	1.43±0.28	0.89±0.14

*$p<0.05$, **<0.01 (Student's t-test)

It thus appears that chronic treatment of rats with H_2S at a dose level below lethality results in alterations in brainstem amino acids. Since the affected aminoacids are also considered putative neurotransmitters, it remains to be determined whether chronic H_2S exposure influences the mechanisms underlying synaptic transmission as well as neuronal excitability.

Supported by the Occupational Health and Safety Heritage Grant Program, Alberta Department of Community and Occupational Health.

Beauchamp, R.O. Jr. et al. (1984) CRC Crit. Rev. Toxicol. 13: 25-97.
Lindroth, P. and K. Mopper. (1979) Anal. Chem. 51, 1667-1674.

THE EFFECT OF TOPICALLY APPLIED MUSTARD OIL ON RESPONSES OF NOCICEPTIVE DORSAL HORN NEURONES IN THE RAT

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The cutaneous application of mustard oil (3-isothiocyanatoprop-1-ene) produces a burning sensation. Woolf & Wall (1986) concluded, from recordings on a small population of single C and A-delta afferent fibres, that it selectively activates the C-fibres. However, Heapy et.al. (1986), using a multi-fibre recording, concluded that there was also an activation of A-delta fibres. Recently we have shown (Pini & Ryall 1986; Harris & Ryall 1986) that noxious radiant heat excites or inhibits the firing rate of convergent dorsal horn neurones. We therefore applied mustard oil to the receptive fields of the nociceptive dorsal horn neurones to study this further.

Nineteen male Wistar rats, anaesthetised with thiobutabarbital (100-120 mg/kg i.p., supplemented by i.v. administration as required) were spinalised. Extracellular recordings were obtained with glass microelectrodes from 25 lumbar dorsal horn neurones with identified receptive fields on which mustard oil (10 or 20% dissolved in mineral paraffin oil) was tested by topical application to glabrous or hairy skin.

On glabrous skin of the foot, mustard oil had no effect on 2 cells activated by noxious heat or on 6 cells which were inhibited.In contrast, on the hairy skin of the leg, mustard oil excited the neurones and their was no correlation with excitation or inhibition by noxious heat (Table 1). The occurrence of inhibition to noxious heat on hairy skin was similar to its occurrence when glabrous skin is heated. There was a delay of 30-60 s before the onset of excitation by 10% mustard oil and a duration of about 3-5 min. The delay was shorter and the duration longer with 20%. Following the excitation by mustard oil of the heat inhibited cells, the inhibitory responses were still present.

Table 1. Effect of topically administered mustard oil on dorsal horn cells.

	Effect of mustard oil (No. cells)				
	10%	20%	10%	20%	
	Excitation		No effect		Totals
Effect of heat					
Excitation	3	3	1	0	7
Inhibition	1	5	3	1	10
Totals	4	8	4	1	17

The short duration response to mustard oil probably corresponds to the A-delta component of the response to mustard oil described by Heapy et. al.. The failure to detect inhibition after mustard oil application to regions of skin which evoked inhibition, probably mediated by C-fibres, may be due to the masking of the effect by A-delta mediated excitation.

Studies supported by the Wellcome Trust.

Harris, N.C. & Ryall, R.W. (1986) Br. J. Pharmac., **89**, 803P.
Heapy,C.G., Jamieson, A. & Russell, N.J.W. (1987) Br.J.Pharmac.,**90**, 164P.
Pini, A. & Ryall, R.W. (1986) J. Physiol., **378**,42P.
Woolf, C.J. & Wall, P.D. (1986) J. Neuroscience, **6**, 1433-1442.

MEDIATORS OF IGE-DEPENDENT LOCAL ANAPHYLACTIC REACTIONS IN RABBIT SKIN

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We have investigated the mechanisms involved in passive cutaneous anaphylactic reactions (PCA) in the rabbit. IgE antibodies were raised in rabbits against bovine gamma globulin (BGG) by immunisation with BGG (1.5µg dose in 0.5ml aluminium hydroxide gel, s.c) at monthly intervals for 6 months (Kravis & Zvaifler, 1974). Contaminating IgG antibodies were removed from pooled antisera by passing through a column of protein-A Sepharose. Doses of antisera at 3, 10 and 30 times dilution were injected intradermally (i.d) in 0.1ml volumes into the shaved dorsal skin of rabbits. Results presented are for 3 times dilution. This was followed after a fixation period by intravenous (i.v) ^{125}I-human serum albumin and superimposed i.d injections of BGG (1µg/0.1ml) with or without pharmacological agents. Animals were killed with an overdose of barbiturate 30 minutes later and local oedema responses calculated in terms of µl of plasma.

The effect of varying the interval between superimposed antibody and antigen injections was investigated. No responses could be elicited with an interval of 0 or 6 hours. Responses were maximal with a 48–72 hour fixation period and either of these times was used in subsequent experiments. Responses at 24 and 240 hours were approximately 50% of maximal. Unlike our previous results with Arthus reactions in the rabbit which were effectively suppressed by indomethacin (Williams et al, 1986), injections of indomethacin (10^{-8} moles) mixed with the BGG only partly inhibited the PCA, i.e. by 19± 5%, while responses to bradykinin (Bk) + arachidonic acid (10^{-10} moles and 3 x 10^{-9} moles, respectively) were inhibited by 81±8% (means ± s.e.mean for 6 rabbits). BW 755C (10^{-8} moles) did not significantly suppress the PCA (10±3% reduction) but suppressed oedema induced by Bk + arachidonic acid (doses as before) by 87±3% (n=6 rabbits). Thus, potentiation of oedema by endogenous prostaglandins does not appear to be important in the PCA reaction. Local antihistamines were poor inhibitors of the PCA: mepyramine (3 x 10^{-9} moles) reduced oedema by 13±5%, whereas responses to histamine + PGE_2 (10^{-8} moles and 3 x 10^{-10} moles respectively) were suppressed by 92±3% (n=6 rabbits). Cimetidine (3 x 10^{-7} moles) did not increase suppression when combined with mepyramine. The PAF antagonists L-652731 (5 x 10^{-8} moles) and 48740 RP (10^{-6} moles) produced marked suppression of responses to PAF + PGE_2 (10^{-9} moles and 3 x 10^{-10} moles respectively) and the Arthus reaction (Hellewell & Williams, 1986) but had no effect on the PCA. The kinin formation inhibitor Trasylol (1µg) effectively suppressed responses to Kallikrein + PGE_2 (500ng and 3 x 10^{-10} moles respectively) but had no effect on the PCA. Disodium chromoglycate (100µg) was also without effect. Colchicine (1mg/kg i.v) was the only agent tested to cause marked suppression of the PCA (54±9%, n=5 rabbits). Control responses to F-met-leu-phe + PGE_2 (5 x 10^{-11} moles and 3 x 10^{-10} moles respectively) were reduced by 84±5% in these experiments whereas responses to Bk + PGE_2 (doses as before) were reduced by only 15±2%. When the local anaphylactic reaction was established in the peritoneal cavity no C5a was detectable in the exudate, using radioimmunoassay (limit of detection ∿5ng/ml).

Thus, the results indicate that none of the established mediators is involved in the rabbit PCA. We have observed accumulation of ^{111}In-labelled neutrophils in the PCA and we are investigating the possibility that an unidentified chemoattractant may be involved in oedema formation.

This work was supported by the MRC and Pfizer Research, Groton, USA.

Hellewell, P.G. & Williams, T.J. (1986), J.Immunol., 137, 302–307.
Kravis, T.C. & Zvaifler, N.J. (1974), Int.Arch.Allergy, 46, 60–71.
Williams, T.J., Hellewell, P.G. & Jose, P.J. (1986), Agents Actions, 19, 66–72.

THE PIG CORONARY ARTERY IS MORE RESPONSIVE TO ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) THAN THE RABBIT AORTA

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EDRF activity differs markedly in different arteries and appears to be greatest in coronary arteries (Griffith et al., 1984; Angus et al., 1986; Collins et al., 1986). These differences could be due to variation in the amount of EDRF released by different arteries or variation in the response of the smooth muscle to EDRF. We have compared the smooth muscle sensitivity to EDRF of the rabbit aorta (RA) and the pig coronary artery (PCA) using a bioassay cascade system as previously described (Griffith et al., 1984). De-endothelialised rings (2-3 mm wide) of RA and PCA were mounted in parallel for isometric tension recording, and superfused with Holman's solution containing flurbiprofen 10^{-5} M, gassed with 95% O_2, 5% CO_2, flow rate 2 ml/min. In some experiments the Holman's solution was passed through the lumen of a 2 cm length of PCA with an intact endothelium which thus served as a donor for EDRF, which was released by the calcium ionophore A23187 (10^{-7} M). A23187 itself did not alter constrictor or dilator responses of the recipient vessels. The de-endothelialised recipient rings were pre-contracted with 5 hydroxytryptamine (5HT) to 95% of the maximum constrictor response to 5HT (ie 3×10^{-6} M for both arteries). The amount of EDRF perfusing the recipient arteries was altered by varying the transit time of the perfusate from the donor vessel, by this means the concentration-response relationship of the recipient arteries to EDRF was established. The maximum EDRF concentration was considered as unity and all other concentrations (calculated from a half-life of c.10 sec in this system) expressed proportionately. Concentration-responses of the recipient arteries were also determined for sodium nitroprusside (NP) which like EDRF relaxes vascular smooth muscle by stimulation of soluble guanylate cyclase.

Transit Time	5s	10s	15s	30s
Relative EDRF Concentration	1.0	0.7	0.5	0.18
PCA	94 ± 5 (11)	58 ± 7 (5)	44 ± 11 (5)	24 ± 13 (4)
RA	45 ± 4 (11)	30 ± 5 (5)	17 ± 3 (5)	11 ± 3 (4)
Ratio	2.09:1	1.93:1	2.59:1	2.18:1

The table shows the percent relaxation (mean ± SE; (n))and the PCA:RA response ratio obtained with varying concentrations (ie transit times) of EDRF. The PCA:RA ratio was similar at all EDRF concentrations studied. This differential response of RA and PCA to EDRF was also shown to NP and was of a similar order of magnitude ie. the PCA giving approximately twice the response of the RA at any concentration. Preliminary studies also show that the PCA is more responsive than the RA when using endothelialised RA as an EDRF donor.

These studies show that differences in EDRF activity in different arteries are due in part to differences in the smooth muscle responsiveness to EDRF.

Angus et al. (1986) Br. J. Pharmac. 88, 767-777.
Collins et al. (1986) J. Cardiovasc. Pharmac. 8, 1158-1162.
Griffith et al. (1984) Nature 308, 645-647.

This study was supported by the British Heart Foundation.

STIMULATION OF CHLORIDE SECRETION BY U46619 IN GUINEA-PIG ISOLATED GASTRIC MUCOSA IS MEDIATED BY THROMBOXANE RECEPTORS

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We have previously shown that prostaglandin E_2 stimulated electrogenic chloride secretion in guinea-pig isolated gastric mucosa (Spraggs & Bunce, 1985), and have suggested that this observation provides a mechanism for the stimulation of a NaCl-rich secretion by prostaglandins in mammalian stomach in vivo (Bunce, 1985). The stable thromboxane A_2-mimetic, U46619 (11α, 9α-epoxymethano-prostaglandin H_2), also stimulates gastric NaCl secretion in vivo (Bunce & Clayton, 1985) and we have therefore investigated the effect of U46619 on transport mechanisms in guinea-pig gastric mucosa in vitro, and have also characterised the prostanoid receptor type mediating this response.

Guinea-pigs were anaesthetised by halothane inhalation, the gastric mucosa was stripped from the overlying muscle layers and mounted in Ussing chambers. The tissues were bathed bilaterally with Krebs-Henseleit solution at 37°C and gassed with $95\%O_2/5\%CO_2$. The mucosa was clamped at zero transepithelial potential by application of short-circuit current (SCC) which was recorded continuously. Tissues were pretreated with indomethacin (1μM serosally); all other drugs were added bilaterally.

U46619 caused dose-related increases in SCC with an EC_{50} (95% confidence limits) value of 38 (16-96) nM (n=6) and a maximum response of $71\pm8\mu Acm^{-2}$ (mean ± s.e. mean, n = 6) at 1μM U46619. The ionic basis of this response was determined by ^{36}Cl and ^{22}Na flux studies. Under resting conditions net secretion of ^{36}Cl ($3.37\pm0.23\mu Eqcm^{-2}/30min$) was not significantly different from SCC ($3.71\pm0.18\mu Eqcm^{-2}/30min$, $P>0.05$) while the net absorption of ^{22}Na ($0.39\pm0.20\mu Eqcm^{-2}/30min$) was significantly smaller than SCC ($2.91\pm0.14\mu Eqcm^{-2}/30min$, $P<0.001$). U46619 (1μM) caused a significant increase in SCC ($5.18\pm0.09\mu Eqcm^{-2}/30min$, $P<0.001$) and net ^{36}Cl secretion ($6.28\pm0.87\mu Eqcm^{-2}/30min$, $P<0.001$); these values were not significantly different. U46619 also significantly increased net ^{22}Na absorption ($1.50\pm0.22\mu Eqcm^{-2}/30min$, $P<0.01$) but again this was significantly smaller than SCC ($4.84\pm0.25\mu Eqcm^{-2}/30min$, $P<0.001$).

The prostanoid receptor mediating the effect of U46619 on SCC was investigated using the thromboxane receptor antagonist, AH23848. AH23848 (0.03,0.1,0.3μM) caused parallel rightward shifts of the U46619 concentration-response curve with no depression of the maximum response. A Schild plot derived from these data gave an estimated pA_2 value of 8.4 with a slope of 0.98; these results agree well with those obtained for antagonism by AH23848 of U46619-induced contraction of dog saphenous vein (Brittain et al. 1985). AH23848 (1μM) did not affect the concentration-response (SCC) curve to PGE_2.

These results show that U46619 increased SCC in guinea-pig isolated gastric mucosa and that this response was due predominantly to the electrogenic secretion of chloride ions. This effect of U46619 was mediated by thromboxane receptors.

Brittain, R.T. et al. (1985). Circulation 72, 1208-1218.
Bunce, K.T. (1985). Br. J. Pharmac. 86, 815P.
Bunce, K.T. & Clayton, N.M. (1985). Br. J. Pharmac. 86, 421P.
Spraggs, C.F. & Bunce, K.T. (1985). Gut 26, A1147-A1148.

EFFECTS OF CORTICOSTEROIDS ON MOTILITY OF ISLOATED OVINE URETERAL RINGS

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The nonsteroidal anti-inflammatory drug (NSAID) indomethacin has been shown to inhibit rhythmic activity in isolated ureteral preparations (Thulesius & Angelo-Khattar et al 1985). The pain relief produced by NSAID's in renal colic may partly be attributed to this spasmolytic action. The potential use of glucocorticosteroids in renal colic is yet unknown. We studied the effect of corticosteroids on the motility of ureteral preparations.

Ureteral rings (4 mm) from freshly slaughtered sheep were suspended in 10 ml organ baths filled with Krebs-Henseleit solution and aerated with 95% oxygen and 5% carbon dioxide. Isometric tension was recorded. After a latency of 30 min phasic rhythmic contractions started and could be maintained for 5 h. Addition of NSAID's and corticosteroids reduced frequency and amplitude and finally stopped contractions. Drug effect was measured by determining the interval (in min) until cessation of rhythmic activity.

Dexamethasone (10^{-8} - 10^{-4}M) and methylprednisolone (10^{-7} - 10^{-4}M) dose dependently block ureteral motility. Hydrocortisone has an inhibitory action only at a high concentration of 10^{-4}M. The relative potencies of the three corticosteroids and two NSAID's, indomethacin and diclofenac Na are shown in the table below. Pretreatment of ureteral strips with the protein synthesis inhibitor cycloheximide (10^{-6}M) abolishes the peristalsis inhibitory action of the corticosteroids. This suggests that the effect of corticosteroids on ureteral motility is mediated via the synthesis of the anti-phospholipase protein, lipocortin, responsible for the anti-inflammatory effect of corticosteroids (Di Rosa, et al, 1984).

	indomethacin	diclofen. sodium	dexamethasone	methylprednisolone	hydrocortisone
EC_{50} (M)	2.1×10^{-6} n = 15	1.1×10^{-6} n = 10	2.8×10^{-6} n = 8	1.2×10^{-5} n = 8	2.8×10^{-4} n = 6
Relative potency	1	1.88	0.75	0.17	0.007

Thulesius, O. & Angelo-Khattar, M. Acta Pharm. et toxicol (1985) 56, 298
Di Rosa, M. et al. Prostaglandins (1984) 28, 441

Supported by grant No. MR 015 Kuwait University

RELEASE OF ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) IS INHIBITED BY 8-BROMO-CYCLIC GMP

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EDRF (Rapoport & Murad, 1983) and the nitrovasodilators (Murad et al., 1979) relax vascular smooth muscle by stimulating soluble guanylate cyclase thus elevating cyclic GMP (cGMP), which in turn reduces phosphatidyl inositol hydrolysis (Rapoport, 1986) and calcium fluxes (Collins et al., 1986). Endothelial cells contain both the soluble and particulate forms of guanylate cyclase (Martin & White, 1987). We have therefore investigated the effects of 8-bromo-cGMP, a lipid soluble analogue of cGMP, on EDRF release induced by acetylcholine (ACh) and substance P (SP). Isometric tension recordings were made from rings of rabbit aorta, with or without intact endothelium, bathed in Kreb's solution at 37° and preconstricted to c.60% of maximum with phenylephrine (PE) (10^{-7} M to 10^{-6} M). When required, these rings were pretreated with 5×10^{-5} M 8-bromo-cGMP added 10 min prior to the PE.

Endothelium-dependent relaxation induced by ACh (3×10^{-8} M to 5×10^{-6} M) or SP (3×10^{-10} M to 10^{-6} M) were inhibited by 8-bromo-cGMP, shifting the concentration-response curves to the right (3.8 fold for ACh, 6.2 fold for SP) and depressing the maximum response (from 87 to 67% relaxation for ACh, and 63 to 33% relaxation for SP).

In endothelium-denuded preparations, relaxations to sodium nitroprusside were, however, unaffected by 8-bromo-cGMP pretreatment. These results show that inhibition of the endothelium-dependent relaxation by 8-bromo-cGMP was due to reduced EDRF release, and not a reduced vascular smooth muscle response to EDRF. This suggests a role for cGMP in regulating EDRF release, perhaps through a negative feedback mechanism.

Collins, P. et al. (1986). J. Physiol., 381, 427-437.
Martin, W. and White, D.G. (1987). Br. J. Pharmacol. Proceedings December 1986 Meeting.
Murad, F. et al. (1979). Adv. Cyclic. Nucleotide Res. 11, 175-204.
Rapoport, R.M. (1986). Circ. Res., 58, 407-410.
Rapoport, R.M. and Murad, F. (1983). Circ. Res. 52, 352-357.

This work was supported by the British Heart Foundation.

METABOLISM OF PROSTAGLANDIN D_2 TO 9α, 11β-PGF_2 AND SUBSEQUENT TRANSFORMATION IN RAT, RABBIT AND GUINEA-PIG

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Prostaglandin (PG) D_2 is a putative inflammatory mediator in man, but little is known of its metabolism. One initial reaction involves stereoselective NADPH-dependent reduction of the C-11 keto function to yield 9α,11β-PGF_2 (Liston & Roberts, 1985), a metabolite which itself has potent actions. Our aim was to compare the distribution of PGD_2 11-ketoreductase (11KR), PGE_2 9-ketoreductase (9KR) and 15-hydroxyprostaglandin dehydrogenase (15-PGDH) in organs from rabbit, rat and guinea-pig.

Cytosolic supernatants were prepared from liver, lung, kidney and caecum and PG metabolism studied by radio-t.l.c. using 60 min incubations at 37°C with 2 μg/ml substrate and 0.1 μCi tritiated PG, supplemented with 4 mM NAD^+ for the 15-PGDH studies (substrate $PGF_{2\alpha}$) or an NADPH regenerating system in the 11KR and 9KR studies. The identity of the products was confirmed by C18 reversed phase hplc using a mobile phase of 32.8% v/v acetonitrile in 0.017M phosphoric acid at 1 ml/min, and also by electron impact GC/MS.

Metabolism of PGD_2 to 9α,11β-PGF_2 proceeded rapidly in 100,000 g supernatant fractions of guinea-pig kidney and liver and rabbit liver (specific activities 55 ± 1, 28 ± 1, 27 ± 1 pmol/min/mg, respectively, n = 8), but was less than 5 pmol/min/mg in 8 of the other 9 systems, the exception being guinea-pig caecum (12 ± 1 pmol/min/mg, n = 5). The sole product of the reaction was 9α,11β-PGF_2 as identified by hplc and GC/MS using conditions which completely resolve the 11α epimer (i.e. $PGF_{2\alpha}$). 9-ketoreductase activity was high only in rabbit liver and kidney (sp. act. 13.4 ± 0.3 and 14.2 ± 0.1, n = 4), showing its distribution to be distinct from 11-KR. In incubations supplemented with NAD^+, 9α,11β-PGF_2 was itself effectively metabolised in several organs (e.g. sp. act. 14 ± 1 and 25 ± 2 pmol/min/mg in guinea-pig liver and rat caecum, n = 8), at about one fifth of the rate of $PGF_{2\alpha}$ by a sulphasalazine analogue inhibitable enzyme to yield two metabolites, one of which co-eluted with 13,14-dihydro-15-keto-9α,11β-PGF_2. In human lung supernatants, presumptive GC/MS evidence was also obtained for the formation from 9α,11β-PGF_2 of 15-keto-9α,11β-PGF_2 with fragment ions at m/z 539 (M^+), 508 (loss of $.OCH_3$), 449 (loss of Me_3SiOH), 418 (loss of Me_3SiOH and $.OCH_3$), 468 (loss of $.C_5H_{11}$). These data indicate that 9α,11β-PGF_2 is readily inactivated by the 15-PGDH route.

In conclusion we have mapped the comparative activity of PGD_2-11KR in 4 organs from 3 species and shown the enzyme to be distinct from the 9KR enzyme which metabolises PGE_2. Furthermore, 9α,11β-PGF_2 (the initial product of PGD_2 metabolism) was itself readily metabolised by NAD^+-dependent 15-PGDH.

Supported by the Asthma Research Council.

Liston, T.E. & Roberts, L.J. (1985) Proc. Natl. Acad. Sci. USA, 82, 6030.

EVIDENCE FOR THE EXISTENCE OF THREE SUBTYPES OF PGE_2 SENSITIVE (EP) RECEPTORS IN SMOOTH MUSCLE

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Prostaglandin (PG) E_2-sensitive (EP-)receptors can be divided into EP_1 and EP_2 subtypes, the prostanoid receptor blocking drugs, SC-19220 and AH6809, specifically antagonising EP_1 but not EP_2-receptors (Coleman et al., 1980a, 1985; Kennedy et al., 1982). This subdivision of EP-receptors is supported by data with prostanoid agonists, where the PGE analogues, sulprostone and AY23626, are highly selective agonists at EP_1 and EP_2-receptors respectively in a wide range of preparations (Coleman et al., 1986). However, we now describe data obtained with sulprostone and AY23626 on chick ileum (ChI), a preparation previously believed to contain EP_2-receptors (Coleman et al., 1980b), which are inconsistent with the receptors in this preparation being EP_2, and suggest the need for further subdivision of EP-receptors.

We report results obtained with sulprostone and AY23626 on guinea-pig fundus (GPF) and cat trachea (CT), which contain EP_1 and EP_2-receptors respectively (Kennedy et al., 1982), as well as on ChI. PGE_2 was the standard agonist in all experiments, and the experimental design is as previously described (Kennedy et al., 1982). The results are summarized in the Table.

Preparation	Receptor	PGE_2[1] EC_{50}(nM)	Equipotent Concentration (PGE_2=1)[1]	
			Sulprostone	AY23626
GPF	EP_1	7.6 (4.8-11.9)	2.6 (1.8-3.7)	430 (245-754)
CT	EP_2	19.7 (12.2-31.2)	>7,000	1.2 (0.5-3.0)
ChI	EP_2?	10.0 (7.1-14.4)	0.7[2] (0.4-0.9)	5.5 (3.6-8.5)

1. geometric means (95% C.L.), n>4. 2. lower maximum response than PGE_2.

AY23626, while only a weak agonist on GPF, was potent on both CT and ChI, consistent with CT and ChI containing a common receptor type. However, sulprostone, although inactive as an agonist on CT, was highly potent on ChI. We have previously shown that sulprostone has no agonist (or antagonist) activity in a range of other EP_2-receptor containing tissues (Coleman et al., 1986). The actions of sulprostone on ChI are not mediated by EP_1-receptors, since like those of PGE_2, they are resistant to blockade with either SC-19220 (100μM) or AH6809 (3μM) in this preparation. We conclude therefore, that whilst the agonist profile of AY23626 is consistent with the simple subclassification of EP-receptors into EP_1 and EP_2-subtypes, the data with sulprostone suggest that it may be necessary to further subdivide EP_2-receptors.

Coleman, R.A. et al. (1980a) Br. J. Pharmac. 69, 266P-267P.
Coleman, R.A. et al. (1980b) Br. J. Pharmac. 70, 89-90P
Coleman, R.A. et al. (1985) Br. J. Pharmac. 85, 273P
Coleman, R.A. et al. (1986) Adv. Prostaglandin Thromboxane Leukotriene Res. (in press).
Kennedy, I. et al. (1982) Prostaglandins 24, 667-689.

PROSTACYCLIN AND NITRIC OXIDE: INTERACTIONS AS INHIBITORS OF AGGREGATION OF HUMAN PLATELETS

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Prostacyclin and endothelium-derived relaxing factor (EDRF) are unstable vasodilatory factors generated by vascular endothelium (Moncada et al., 1976; Furchgott and Zawadzki, 1980). Recently Furchgott (1986) suggested that nitric oxide (NO) might be EDRF. Nitric oxide and EDRF have been shown to inhibit platelet aggregation (Mellion et al. 1981; Azuma et al. 1986). We have now studied the effect of prostacyclin and/or NO (0.1% v/v in He-deoxygenated water) on aggregation of human platelets.

Platelet aggregation in platelet-rich plasma (PRP) or in washed platelets (WP) (Radomski and Moncada, 1983) was measured in a Payton dual channel aggregometer.

Platelet aggregation-induced by ADP (8-10 μM), collagen (1 μg/ml), thrombin (0.02U/ml) or U-46619 (1 nM) was inhibited in a concentration-dependent manner by NO with IC_{50}s ranging from 0.42 to 0.85 μM in PRP and from 0.24 to 0.31 μM in WP. In addition, when a non-inhibitory concentration of prostacyclin (0.1 nM) was incubated for 1 min before addition of NO significant ($p < 0.005$, n=10) potentiation of the anti-aggregating activity of NO was observed (Table 1).

Table 1 Potentiation of anti-aggregating activity of NO by prostacyclin in WP.

	IC_{50} (μM) (n=10)	
Agonists	NO	NO + prostacyclin
ADP	0.28 ± 0.04	0.05 ± 0.01
Collagen	0.25 ± 0.03	0.06 ± 0.02
Thrombin	0.31 ± 0.04	0.08 ± 0.02
U-46619	0.24 ± 0.03	0.05 ± 0.01

Both NO (0.12 - 1.25 μM) and prostacyclin (0.1-60 nM) also induced disaggregation of platelets aggregated with collagen. Inhibitors of c-GMP phosphodiesterase (M&B 22948 1 μM) or of c-AMP phophodiesterase (HL 725 1 fM) selectively potentiated the anti-aggregating activity of NO or prostacyclin respectively.

Thus, NO is a potent inhibitor of platelet aggregation. In addition, prostacyclin and NO act synergistically as inhibitors of platelet aggregation. If NO is EDRF the above interactions may be significant in terms of platelet vessel wall homeostasis.

Moncada, S., et al. (1976) Nature, 263:663.
Furchgott, R.F. & Zawadzki, J.V. (1980) Nature 288:373.
Furchgott, R.F. (1987). In: Mechanisms of Vasodilatation IV, ed. Vanhoutte, P.M., Raven Press, New York, in press.
Radomski, M. & Moncada, S. (1983). Thromb. Res., 30:383.
Mellion, B.T. et al. (1981). Blood, 56:946.
Azuma, H., et al. (1985). Br. J. Pharmac., 88, 411.

ARE CIMETIDINE AND RANITIDINE PROARRHYTHMIC?

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Histamine is a vasoactive amine found in mast cells throughout the body. Cardiac effects of H_2 stimulation include increased sinus rate and contractility, and enhanced automaticity (Levi et al, 1976). Under ischaemic conditions it has been suggested that these actions may be proarrhythmic and that the use of H_2 receptor antagonists could provide antiarrhythmic protection (Cameron et al, 1983). Bradycardia has been the cardiovascular complication most frequently associated with the use of these drugs, but there have also been reports of serious cardiac arrhythmias (Cohen et al, 1979) and even sudden death (Shaw et al, 1980). We have developed a chronic canine model of myocardial infarction where re-entrant arrhythmias may be initiated by programmed electrical stimulation (PES). Using this model in a placebo controlled study we have investigated the proarrhythmic and electrophysiological effects of cimetidine and ranitidine. After anaesthesia with sodium methohexitone, 10 mg/kg, artificial ventilation with 1.5% halothane and thoracotomy, coronary artery ligation was performed in adult greyhounds (Harris, 1950). Myocardial pacing wires were placed within and adjacent to the infarcted area. 7,14,21 and 28 days later the conscious dogs underwent PES. Ventricular pacing was performed using bipolar pulses of 4 msec duration at twice diastolic threshold. Up to three extrastimuli were then introduced until an arrhythmia was produced (sustained ventricular tachycardia or a reproducible arrhythmia of 4 or more ectopic beats), or the protocol was exhausted.
Groups of 6 dogs in which reproducible ventricular tachycardias had previously been produced but in which no arrhythmia could be initiated on this occasion, were randomly allocated to receive increasing (0.5, 1.0 etc mg/kg) intravenous doses of cimetidine, ranitidine or placebo. 5 mins after each dose the dog was rechallenged with the stimulation setting which had produced the maximal ectopic response. Results were ranked for no change, induction of arrhythmia or death, and compared with placebo using the non-parametric Mann-Whitney U test. PR and QT_c intervals, QRS duration, effective and functional refractory periods, blood pressure (BP) and heart rate (HR), were measured before and after each treatment.
In the placebo group, 4/6 dogs remained unchanged, 1 developed an arrhythmia and 1 died. With cimetidine, 4/6 dogs remained unchanged, 1 developed an arrhythmia (4 mg/kg) and 1 died (0.5 mg/kg). With ranitidine, 3/6 remained unchanged and 3 died ($P=0.24$) (1.0 mg/kg, 4.0 mg/kg, 16.0 mg/kg), PR, QT_c, QRS, refractory periods and BP were unaltered in all groups; Mean HR did not change following placebo, however, following cimetidine and ranitidine HR increased ($p<0.05$) compared with placebo (220.0 ± 14.7 {mean ± SEM}, 180.0 ± 31.9, 108.4 ± 5.8 beats/min respectively).
In conclusion, this study failed to demonstrate a significant proarrhythmic effect from the use of intravenous cimetidine or ranitidine at therapeutic doses in a chronic myocardial infarction model. Significant changes in heart rate were apparent after treatment with both drugs when compared with placebo. These changes occurred only at high doses and may be a reflection of drug toxicity.

Levi, R., et al (1976). Fed Proc 35 (8), 1942-1947.
Cameron, J.S., et al (1983). Circulation 68, 111-221 (abstr.).
Cohen, J., et al (1979). Br Med J 2, 768.
Shaw, R.G., et al (1980). Med J Aust 2, 629-630.
Harris, A.S. (1950). Circulation 1, 1318-1328.

AN ANOMALOUS INTERACTION BETWEEN PROPRANOLOL AND ISOPRENALINE

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It has been known for some time that isoprenaline, terbutaline or salbutamol increase the sensitivity of guinea-pigs to inhaled histamine (Conolly et al., 1971). More recently, it has been established that isoprenaline increases the hyperreactivity of airways due to infusion of platelet activating factor (Mazzoni et al., 1986). Hence, we have been interested to ascertain whether such unexpected observations might be accounted for by induction of hyperreactivity following exposure to a beta-adrenoceptor agonist.

Anaesthetised (100 mg/kg i.p. phenobarbitone and 30 mg/kg i.p. pentobarbitone), ventilated (1Hz, 10ml/kg) guinea-pigs have been used to monitor airway resistance (R_L cm H_2O/l/sec) and compliance (C_{dyn} ml/cm H_2O), which were calculated from tracheal airflow and transpulmonary pressure, using a Buxco respiratory analyser. Hyperreactivity was estimated as the difference between the effect upon airway resistance of intravenous bombesin (240 ng/kg) before, and 20 minutes after, infusion of isoprenaline, propranolol or mixtures thereof. In agreement with previous observations (Ney et al, 1979), infusion of propranolol (1-100 ug/kg/hr) induces airway hyperreactivity. We now report that infusion of isoprenaline (1-100 ug/kg/hr) induces airway hyperreactivity of similar magnitude (Table). Induction of hyperreactivity by isoprenaline is not inhibited by propanolol. Thus, an increased response to bombesin (R_L) is observed after infusion (10 ug/kg/hr) of isoprenaline (115±27, n=10) or propanolol (166±41,n=10) and when administered together, the response to bombesin is increased (228±79,n=5). In isoprenaline (100 ug/kg/h) treated animals, relaxation of 53±4% and 85±5% was achieved by a bolus dose of isoprenaline (0.1-1.0 ug/kg), whereas in propanolol (100 ug/kg/h) treated animals the response was diminished (26±4 and 73±7%). These effects of isoprenaline and propranolol upon airway resistance are accounted for by beta-adrenoceptor occupancy, but effects upon airway hyperreactivity are not readily explained by such a mechanism.

Drug	Dose (ug/kg) 1	10	100
Isoprenaline	72 ± 21	115 ± 27	282 ± 42
Propranolol	115 ± 27	166 ± 41	310 ± 52

Conolly, M.E. et al (1971) Br. J. Pharmac. 43, 389-402
Mazzoni, L. et al (1985) Br. J. Pharmac. 86, 571P
Ney, U.M. (1983) Br. J. Pharmac. 79, 1003-1009

A COMPARATIVE STUDY OF THE α-ADRENOCEPTOR ANTAGONISM OF CORYNANTHINE. PRAZOSIN AND YM-12617 IN THE RAT ISOLATED THORACIC AORTA

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Downing *et al.*, (1983) reported that prazosin produced a non-competitive antagonism of the initial component of responses of the rat isolated thoracic aorta to (-)-noradrenaline (NA); an effect observed with other potent aminoquinazoline-type antagonists. The selective and potent α_1-adrenoceptor antagonist YM-12617 (5-(2-((2-(2-ethoxyphenoxy)ethyl)-amino)propyl)-2- methoxybenzene sulfonamide HCl - Honda *et al.*, 1985) provides an opportunity to test the importance of potency *per se* in this unusual effect of prazosin.

2-3mm wide denuded rings of the rat isolated thoracic aorta from Male Wistar rat (240-270g) were placed under a resting tension of 1.5g in Krebs solution with 1μM propranolol, gassed with 95% O_2 5% CO_2 and maintained at 37°C. Responses to 3μM NA were elicited 5 min after exposure to Ca^{++}-free Krebs with 0.5mM EGTA and, following attainment of a stable response, 2.5mM Ca^{++} was readded. This was repeated in the presence of equieffective concentrations of the antagonists (determined from their effect on cumulative NA concentration-response curves). All responses are expressed as a percent of the response after the readdition of Ca^{++}.

Prazosin and YM-12617 were equipotent (pA_2 = 10) and both were ~ 200-fold more potent than corynanthine (pA_2 = 7.6). In the presence of Ca^{++}-free Krebs, responses to 3μM NA were characterised by an initial transient contraction (ITC) followed by relaxation to a stable response (SC). The addition of 2.5mM Ca^{++} was associated with a further contraction (100%) and the time to 30% of maximum (T_{30}) was determined. The effect of "equieffective" concentrations of the three antagonists on SC and T_{30} was surmountable (Table 1). In marked contrast to corynanthine, however, the effect of both YM-12617 and prazosin on the ITC was unsurmountable.

Table 1: The effect of equieffective concentrations of YM-12617, Prazosin and Corynanthine on responses in Ca^{++}-free Krebs (n=4-5).

	3μM NA			100μM NA		
	ITC	SC	T_{30}	ITC	SC	T_{30}
Control	34.6±1.3	7.1±0.6	27.6±4.4			
Cory 1μM	22.4±1.8	3.7±0.6	39.6±5.8	33.8±3.0	7.9±1.2	25.2±4.8
Praz 5nM	1.4±1.6	5.4±1.2	38.2±7.5	1.4±1.6	7.9±0.9	25.5±4.1
YM 5nM	5.1±3.6	4.2±0.6	34.5±7.6	7.0±4.9	8.2±2.8	25.5±7.8

In conclusion, the effect of prazosin on the inital component of contractions in the rat aorta is not peculiar to potent prazosin-type antagonists - potency *per se* is an important determinant. Furthermore, these observations support the view that the slow dissociation of prazosin (consistent with the high potency) from the receptor specifically interferes with the events associated with NA-induced calcium release in the rat aorta (Downing *et al.*, 1986).

Downing O.A. *et al.*, (1983) Br. J. Pharmac. 80, 315-322.
Downing O.A. *et al.*, (1986) Br. J. Pharmac. 89, Proc. Suppl., 841P
Honda K. *et al.*, (1985) N-S Arch Pharmacol. 328, 264-272.

IN VITRO DESENSITIZATION BY NORADRENALINE OF AORTIC BUT NOT MYOCARDIAL α-ADRENOCEPTOR RESPONSES

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Desensitization of β-adrenoceptor mediated responses by agonists is well established (Limas & Limas, 1985), but the effects of high concentrations of catecholamines on α-adrenoceptors has received less attention. This study examines the in vitro desensitization by noradrenaline of cardiac and aortic tissues in the rat. The desensitization of cardiac tissues with phenylephrine is also examined.

Rat left atria, papillary muscles and thoracic aorta were set up in aerated Krebs solution at 32°C. Aorta were cut spirally into a strip (3 x 25mm), set up under 1g tension and isometric tension recorded. Following 120 min incubation with or without noradrenaline (50μM), tissues were washed and cumulative concentratration-response curves to PHE constructed. Cardiac tissues were paced at 1 Hz and isometric developed tension recorded. After 30min equilibration, the stimulator was turned off and the tissues incubated with or without noradrenaline (50μM) for 120 min. After washout tissues were again paced at 1 Hz and cumulative concentration-response curves to isoprenaline (ISO) or PHE (with a maximum ISO concentration added at the end of the experiment) were constructed in the presence of desipramine (1μM) and metanephrine (10μM).

Table 1: EC_{50} values and maximum responses to PHE in control, NA incubated and PHE incubated tissues

	EC_{50} (μM)	M.D.T.(g)	Maximum(% ISO)
Left Atria			
Control	5.9 (3.9 - 8.9)	0.63 ± 0.09	63.3 ± 3.7
NA-incubated	8.7 (4.3 - 17.7)	0.62 ± 0.10	68.2 ± 4.4
PHE-incubated	8.6 (5.1 - 14.3)	0.42 ± 0.05	47.0 ± 7.3*
Papillary Muscle			
Control	3.7 (2.5 - 5.4)	0.88 ± 0.16	57.1 ± 5.7
NA-incubated	8.7 (1.4 - 51.9)	0.95 ± 0.16	57.4 ± 8.2
PHE-incubated	9.1 (2.6 - 31.1)	0.53 ± 0.09	36.9 ± 8.9*

*P<0.05

Incubation of left atria with NA produced a desensitization of responses to ISO with an increase in EC_{50} values from 2.1 (1.1-3.8)nM to 45.4 (22.5 - 91.3)nM (P<0.001). Maximum developed tension (MDT) to ISO was not significantly different between control (0.81 ± 0.19g) and desensitized tissues (0.65 ± 0.08g). Incubation of papillary muscles with NA did not alter ISO EC_{50} values (control 20.2 (9.8 - 41.7); desensitized 38.9 (7.4 - 204.2)nm) but resulted in a reduction in MDT from 1.00 ± 0.13g to 0.67 ± 0.04g (P< 0.05). Incubation of cardiac tissues with NA did not alter responses to PHE (Table 1). Incubation with PHE (500μM) however, reduced the maximum response of cardiac tissues to PHE (Table 1). In contrast to cardiac tissues, incubation of aorta with NA desensitized the tissues to PHE, EC_{50} values were increased from 25(15.8 - 39.4)nM to 215 (85.4 - 540.7)nM (P<0.001). The maximum tension developed to PHE was similar for control (0.38 ± 0.08g) and desensitized aorta (0.33 ± 0.04g).

These results suggest that vascular but not myocardial α-adrenoceptor mediated responses are desensitized by NA. Myocardial α-adrenergic responses may however be desensitized by PHE.

This work is supported by the British Heart Foundation and Bristol-Myers.

Limas, C.J. & Limas, C. (1985) Circ. Res. 55: 524-531.

NO EVIDENCE FOR MORE THAN ONE TYPE OF α_1-ADRENOCEPTOR IN RABBIT PULMONARY ARTERY

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Flavahan & Vanhoutte (1986) have recently suggested that there are two subtypes of alpha-1 adrenoceptor, based largely on the results of Holck et al. (1983) obtained in the rabbit pulmonary artery. These authors found that prazosin and yohimbine antagonised the contractions to clonidine with pA_2 values 1 log unit higher than against methoxamine (Holck et al., 1983). While Flavahan & Vanhoutte (1986) used this as evidence in favour of two subtypes of alpha-1 adrenoceptor, it should be noted that these experiments were carried out in the absence of the uptake blockade and beta-adrenoceptor antagonism thought necessary for characterisation of alpha-adrenoceptors (see Furchgott, 1972). The object of this study is simply to repeat the work of Holck et al. (1983), but in the presence of uptake blockers and propranolol.

Main pulmonary arteries were obtained from male New Zealand White rabbits (2-3 kg), were cut spirally into 4 tissues and placed between a fixed rod and a tension transducer in organ baths at 37°C in Krebs-Henseleit solution containing cocaine (3 uM), corticosterone (30 uM) and propranolol (1 uM). Cumulative concentration-response curves were obtained to phenylephrine or clonidine, and were repeated 2 hrs later after 1 hr exposure to antagonist or vehicle. One tissue from each animal served as a vehicle control to correct for time-dependent change in agonist potency.

Phenylephrine and clonidine contracted the rabbit pulmonary artery with EC50 (concentration producing 50% of maximum contraction) values of 0.52 uM (95% confidence limits of 0.45-0.62 uM) and 0.59 uM(0.43-0.81 uM), respectively (n=20-23). The maximum contraction to phenylephrine was 1.27 ± 0.11 g, whereas clonidine behaved as a partial agonist with a maximum contraction of 0.74 ± 0.10 g.

Mean concentration-response curves were plotted for phenylephrine and clonidine. Prazosin (10 nM) and yohimbine (10 uM) produced approximately parallel shifts in the concentration-response curves for both phenylephrine nad clonidine, allowing for changes in curve shape occurring in vehicle experiments. Prazosin (up to 0.1 uM) and yohimbine (up to 100 uM) did not significantly reduce the maximum contraction to either phenylephrine or clonidine.

Schild plots (Arunlakshana & Schild, 1959) were constructed for the interaction between antagonists and agonists: none of the slopes differed significantly from negative unity. pA_2 values of 5.73 and 5.72 were obtained for yohimbine against phenylephrine and clonidine, respectively. pA_2 values of 8.65 and 8.78 were obtained for prazosin against phenylephrine and clonidine, respectively.

We can conclude from the present results that the rabbit pulmonary artery contains an apparently homogeneous population of alpha-1 adrenoceptors. There is no evidence for subtypes of alpha-1 adrenoceptor in this tissue when experiments are carried out under conditions thought ideal for receptor characterisation. Hence, it is premature at the present moment to suggest subtypes of alpha-1 adrenoceptor.

Supported by RCSI and MRC(I).

Arunlakshana, O. & Schild, H.O. (1959). Br. J. Pharmacol., 14, 48-58.
Flavahan, N.A. & Vanhoutte, P.M. (1986). Trends Pharmacol. Sci., 7, 347-349.
Furchgott, R.F. (1972). in 'Handbook of Experimental Pharmacology Vol. 33, Catecholamines': Blaschko, H. & Muscholl, E. (ed.), pp 283-335. Heidelberg, Springer.
Holck, M.I. et al. (1983). J. Cardiovasc. Pharmacol., 5, 240-248.

CHANGES IN ADRENOCEPTOR NUMBER FOLLOWING CHRONIC NORADRENALINE INFUSIONS IN VIVO IN RABBIT

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We have recently reported the effects of chronic adrenaline (ADR) infusions (0.05 umoles/kg/hr) on adrenoceptor function and number in the rabbit (Deighton et al 1985, 1986). A further study has been carried out using another endogenous catecholamine, noradrenaline (NOR), with the aim of comparing the long term effects of these two agonists on adrenoceptor number.

Male New Zealand white rabbits (2.5 - 3.0 kg) received 10 day infusions of (NOR) (0.09 umoles/kg/hr) via osmotic mini pumps implanted at the femoral vein. Control (cont.) animals received the vehicle (0.1% ascorbate) by the same method. Plasma catecholamines (nM), mean arterial blood pressure (MAP mmHg) and heart rate (HR, beats/min) were measured before, 24 hrs and 10 days into the infusion. On day 11 animals were killed with an overdose of sodium pentobarbitone and blood, kidney, heart and lung removed for radioligand binding studies. [^{3}H] Yohimbine binding (1.25 - 18.75 nM) to alpha adrenoceptors on whole platelets and kidney membranes was quantified as well as [^{125}I] Iodocyanopindolol (ICYP) binding (10 - 150 pM) to beta adrenoceptors on lymphocyte, heart and lung membranes. The maximum number of binding sites, B_{max} (fmoles/mg protein or 10^{8} platelets/ml) and equilibrium binding constant, K_D (nM or pM) were determined by Scatchard analysis. Plasma NOR levels were 3 ± 2 before; 15 ± 11 at 24 hrs and 17 ± 6 at 10 days into infusion (n=12). Significant reductions in B_{max} were observed for kidney, heart and lung without a change on platelets or lymphocytes as summarised in the table.

Radioligand Binding Results (mean ± S.D.)

	[^{3}H] Yohimbine				[^{3}H] ICYP					
	Platelets		Kidney		Lymphocytes		Heart		Lung	
	Cont.	NOR	Cont.	NOR	Cont.	NOR	Cont.	NOR	Cont.	NOR
B_{max}	23±4	28±8	93±38	30*±16	13±4	9±1	40±10	11*± 5	188±46	100*±47
K_D	8±4	11±6	19± 9	13± 7	33±9	35±13	49±24	20*±10	69±17	27*±15

* $p<0.01$ compared to cont. using Wilcoxon test

After 10 days ADR infusion, corresponding values for B_{max} were 10 ± 1, 77 ± 27, 16 ± 6, 22 ± 7 and 115 ± 24 for platelets, kidney, lymphocytes, heart and lung. The trend towards larger falls in receptor number for beta-adrenoceptor binding after NOR infusions may be related to the higher dose of NOR infused. This, however, would not explain the contrasting results obtained for [^{3}H] Yohimbine binding to kidney and platelets after chronic treatment with ADR and NOR. Thus agonist induced changes in receptor number may be tissue specific. Receptor specificity and selectivity of the agonist may also be relevant.

Deighton, NM, Hamilton, CA, Jones CR, Reid JL. (1985). Br.J.Pharmac. 86, 741.
Deighton, NM, Hamilton, CA, Jones,CR, Reid JL. (1986). Br.J.Pharmac. 88, 284.

SUBCLASSIFICATION OF α_1-ADRENOCEPTORS MEDIATING CONTRACTION OF THE CANINE PULMONARY ARTERY

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Analysis of the pA_2 values obtained for prazosin and yohimbine against $alpha_1$-adrenergic contractile responses in blood vessels from rodents and rabbits suggested a subclassification of postjunctional $alpha_1$-adrenoceptors into two subtypes: one with high affinity for prazosin and yohimbine and one with lower affinity for these two antagonists (Flavahan & Vanhoutte, 1986). Only one blood vessel, the pulmonary artery of the rabbit, appeared to contain both $alpha_1$- adrenoceptor subtypes.

The aim of the present study was to analyze the $alpha_1$-adrenoceptors of the pulmonary artery of the dog. No information is yet available on the presence of $alpha_1$-adrenoceptor subtypes on the vascular smooth muscle of this species. Arterial rings were suspended for isometric tension recording in modified Krebs-Ringer bicarbonate solution, gassed with 95% O2/ 5% CO_2 and maintained at 37°C. Experiments were performed in the presence of cocaine (5 x 10-6M), hydrocortisone (3 x 10-5M), propranolol (5 x 10-6M) and rauwolscine (10-7M) in order to inhibit neuronal uptake, extraneuronal uptake, beta- and $alpha_2$-adrenoceptors, respectively.

Prazosin and yohimbine were significantly more potent against responses evoked by phenylephrine (prazosin pA_2 of 9.7, yohimbine pA_2 of 6.8) compared to those produced by methoxamine (prazosin pA_2 of 8.6, yohimbine pA_2 of 6.3). With norepinephrine, low concentrations of prazosin (3 x 10-10M and 10-9M) caused inhibition of the concentration-effect curve, a higher concentration (3 x 10-9M) failed to produce further inhibition, whereas increasing the concentration of the antagonist (to 10-8M and 3 x 10-8M) caused further rightward shifts in the concentration-effect curve to the agonist. The Arunlakshana and Schild plot revealed two components corresponding to pA_2 values of 9.8 and 8.3. Yohimbine (10^{-6}M to 3 x 10^{-5}M) caused concentration-dependent, rightward shifts in the concentration-effect curve to norepinephrine and the pA_2 value was 6.3.

It is concluded that there may be two $alpha_1$-adrenoceptor subtypes present on the vascular smooth muscle of the canine pulmonary artery, each initiating contraction. One receptor subtype has high affinity for prazosin (pA_2 value of 9.7 to 9.8) and yohimbine (pA_2 value of approximately 6.8), may be preferentially activated by phenylephrine and can be termed $alpha_{1H}$. The second subtype has lower affinity for these antagonists (pA_2 values of 8.3 to 8.6 and approximately 6.3, respectively), may be preferentially stimulated by methoxamine and can be termed $alpha_{1L}$. Norepinephrine appears to have similar activity at both receptors.

This work was supported by grants from the National Institute of Health and also from the Mayo Foundation. NAF is presently at the Department of Anatomy, University College, London.

Flavahan, N.A. & Vanhoutte, P.M. (1986) Trends Pharmacol.Sci. 7:347-349

PRESYNAPTIC α_2-ADRENOCEPTORS SENSITIVE TO PHENYLETHYLAMINE STRUCTURE IN HUMAN CYSTIC ARTERY

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Presynaptic alpha2-adrenoceptors which modulate transmitter release from sympathetic nerve terminals have been found in numerous animal tissues as well as in various human blood vessels. Recently a lot of work from a number of laboratories has indicated possible heterogenity of alpha$_2$-adrenoceptors (Vizi et al., 1983). Since investigation of human tissues is potentially of clinical importance, the aim of present study was to determine which type of alpha2-adrenoceptors modulate electrically evoked release of tritiated noradrenaline from human cystic artery.

The influence of alpha2-adrenoceptor agonists and antagonists on release of noradrenaline was studied in human cystic artery preparations, in which transmitter stores were labelled with ^{3}H-noradrenaline. The preparations were stimulated using 2 Hz, 360 shocks each of 1 msec duration. L-Noradrenaline (1 µM), and alpha-methylnoradrenaline (1 µM) significantly reduced, (S_2/S_1=0.27±0.05 and 0.43±0.04 respectively) whilst clonidine, xylazine and guanfacine at 10 uM concentration failed to affect the stimulation evoked release of ^{3}H-noradrenaline. Yohimbine (1 µM), CH-38083 (a new, selective alpha2-adrenoceptor antagonist with berbane structure) [7,8-(methylenedioxi)-14-alpha-hydroxy-alloberbane HCl] (Vizi et al. 1986) (0.1 µM) and prazosin (1 µM) enhanced the evoked release of radioactivity (S_2/S_1 were 2.50±0.19; 2.99±0.32 and 1.48±0.05 respectively). Administering the alpha2-antagonists and prazosin together, we were unable to demonstrate an additive effect. Yohimbine and CH-38083 prevented and prazosin reduced the inhibitory effect of L-noradrenaline or alpha-methylnoradrenaline on release of radioactivity.

Our results suggest that one type of presynaptic alpha2-adrenoceptor modulates the release of noradrenaline evoked by electrical stimulation of human cystic artery. This receptor is sensitive to alpha2-adrenoceptor agonists which have a phenylethylamine structure, but insensitive to imidazolines and quanfacine.

Vizi,E.S., Harsing,L.G.Jr., Gaal,J., Kapocsi,J., Bernath,S. J.Pharm.Exp.Ther. (1986) 238, 701-706.

Vizi,E.S., Ludvig,N., Ronai,A.Z., Folly,G. Eur.J.Pharmacol. (1983) 95, 287-290.