# A CORRELATION BETWEEN N-METHYLASPARTATE ANTAGONIST AND ANTICONVULSANT PROPERTIES OF PCP/SIGMA RECEPTOR AGONISTS

J. Church*, S.N. Davies and D. Lodge, Department of Physiology, Royal Veterinary College, London NW1 OTU.

The phencyclidine (PCP)-like behavioural effects of a series of dissociative anaesthetics and sigma opiates have been correlated with their ability to block the excitatory actions of N-methylaspartate (NMA) in the mammalian CNS (Berry & Lodge, 1985). Such a correlation adds weight to the proposal that reduced excitatory transmission at central synapses utilising NMA receptors may underlie some of the behavioural properties common to compounds acting at the PCP/sigma receptor. The competitive NMA antagonists, 2-amino-5-phosphonopentanoate (AP5) and 2-amino-7-phosphonoheptanoate (AP7), are potent anticonvulsants when administered intracerebroventricularly (Croucher et al. 1982). Because recent reports have linked anticonvulsant properties of dissociative anaesthetics and sigma opiates with their action at PCP/sigma receptors (Hayes & Balster, 1985; Zimmerman et al. 1986), the possibility of a correlation between NMA antagonism and anticonvulsant actions of such drugs has been investigated.

We have therefore extended our previous studies to compare the action of three morphinans (dextrorphan, levorphanol and dextromethorphan), two benzisoquinolines (LY154045 and LY154005), (+) and (-) 2-methyl-3,3-diphenyl-propanolamine (2-MDP) and 5'-methoxy-phenyl-cyclohexylpiperidine (LY189709) with that of the arylcyclohexylamines, dioxalanes and benzomorphans reported previously (Berry & Lodge, 1985). Using microelectrophoretic and i.v. administration on spinal neurones in pentobarbitone-anaesthetised rats and cats (50 and 35 mg/kg i.p. respectively), the drugs were tested as excitatory amino acid antagonists. With the exception of levoxadrol and LY154005, these drugs selectively reduced neuronal responses to NMA rather than to kainate and quisqualate. By comparing effects of two or more drugs on the same neurone, a rank order of potency as NMA antagonists could be made:- phencyclidine > LY189709 > LY154045 > dexoxadrol, etoxadrol, dextrorphan, (-)-2-MDP, (+)-N-allylnormetazocine > ketamine > dextromethorphan > (+)-2-MDP > levorphanol.

This rank order is similar to that obtained for drugs as anticonvulsants in maximal electroshock (MES; Zimmerman et al. 1986) and pentylenetetrazol (PTZ; Hayes & Balster, 1985) tests. Using the Spearman signed rank order statistic, the correlation coefficients were 0.93 and 0.91 for MES and PTZ tests respectively. Unlike AP5 and AP7, PCP/sigma receptor agonists with selective NMA antagonist activity freely cross the blood-brain barrier, and hence may be worth investigating as potential anticonvulsant agents. However, it should be noted that a similar correlation exists between NMA antagonism and PCP discrimination studies in animals (S.C. Berry & D. Lodge, unpublished data) suggesting that the anticonvulsant activity of such NMA antagonists may not be separable from their psychotomimetic, cataleptic and other behavioural effects.

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# RECEPTORS FOR QUINOLINATE ON CORTICAL AND SPINAL NEURONES

D. Lodge & D. Martin*, Department of Physiology, Royal Veterinary College, London NW1 OTU.

Quinolinic acid excites central neurones via N-methyl-D-aspartate (NMDA) receptors but compared to NMDA is less potent on spinal than cortical neurones suggesting regional heterogeneity in quinolinate/NMDA receptors (Perkins & Stone, 1983). To explore this we compared responses of cortical and spinal neurones in vitro to quinolinate and NMDA and tested DL-2-amino-5-phosphono-valerate (2-AP5), a competitive NMDA antagonist (Watkins & Evans, 1981).

Grease seal techniques were used for recording responses of rat cortical slices (Harrison & Simmonds, 1985) and frog hemisected cords to excitatory amino acids (see Martin & Lodge, 1985). Both superfusing media, Krebs and frog Ringer, contained 0.1μM tetrodotoxin but no magnesium. Depolarisations were recorded in response to quinolinate, NMDA, quisqualate and kainate in both preparations but only those to quinolinate and NMDA were sensitive to 2-AP5.

In the cerebral cortex, NMDA and quinolinate both caused dose-dependent depolarisations in the range of 5-80μM and 0.1-2.0mM, ED50s being estimated at 13.5uM and 0.67mM respectively. Schild regression analysis of the antagonism of NMDA and quinolinic acid by 2-AP5 gave pA2 values of 4.85 ± 0.05 and 4.8 ± 0.1 respectively with slopes not significantly different from unity.

On the frog cord NMDA, 10-160μM, produced dose-dependent ventral root depolarisations with an ED50 of 51.3μM, and a $pA_2$ value for 2-AP5 of 5.6 ± 0.05. Quinolinate however produced a diphasic response. In the dose-range, 0.1-2.0mM, hyperpolarising responses were observed which were not reduced by strychnine 200μM, bicuculline 100μM or picrotoxin 100uM nor were they seen in response to L-glutamate, L-aspartate, quisqualate and kainate. But with 4-32mM of quinolinate, depolarisation followed the initial hyperpolarisation. 2-AP5 reduced the depolarising response but enhanced the hyperpolarisation. In view of this complex interaction, rather than attempting to estimate $pA_2$ values for 2-AP5, its potency as an antagonist of quinolinate and NMDA has been compared from shifts in the dose-response curves for depolarisation. For example, at 3.16μM 2-AP5, the dose-ratio was 1.7 + 0.02 for quinolinate and 2.8 ± 0.27 for NMDA. For cortical slices at 10μM 2-AP5 equivalent values were 1.82 ± 0.2 and 1.67 ± 0.04 respectively.

Our results are consistent with the view that NMDA and quinolinate induce depolarisation in the rat cortex via the same receptor type. On frog spinal neurones, quinolinate appears to activate at least two classes of receptor, one mediating 2-AP5-insensitive hyperpolarisation and the other 2-AP5-sensitive depolarisation. How much the hyperpolarising action of quinolinate contributes to its relatively weak action on spinal neurones in vivo (Perkins & Stone, 1983) awaits discovery of a selective antagonist for this novel receptor type.

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# ADENOSINE ANTAGONISTS ENHANCE GLUTAMATE RELEASE AND SPONTANEOUS SYNAPTIC ACTIVITY IN NEURONAL CULTURES.

A.C. Dolphin, S.R. Forda and S.A. Prestwich*. Department of Pharmacology and Clinical Pharmacology, St George's Hospital Medical School, London SW17 ORE.

Adenosine and its analogues potently inhibit synaptic activity in the central nervous system, an effect which is thought to be due to activation of $A_1$ adenosine receptors (Reddington et al, 1982). Presynaptically, exogenously added adenosine analogues inhibit the release of many transmitters, including glutamate (Dolphin and Archer, 1983). In the present study we have examined the role played by endogenous adenosine in the modulation of transmitter release. Two systems have been used (1) release of newly synthesised [$^3$H]-glutamate from cerebellar granule cells in dissociated culture (Dolphin and Prestwich, 1985) and (2) e.p.s.p.s recorded from pyramidal cells in organotypic hippocampal cultures.

The adenosine antagonist 8-phenyltheophylline (8-PT, 10μM) markedly potentiated $K^+$ stimulated [$^3$H]-glutamate release by 131±40% (n=4), and also enhanced basal glutamate release. The agonist (-)-phenylisopropyladenosine ((-)PIA, 2μM), which is relatively selective for $A_1$ receptors reduced by 19±5% the 8-PT-induced enhancement (n=4). Alone, (-)PIA inhibited $K^+$-stimulated glutamate release by 20±5% (n=7), and inhibition by the non-selective adenosine agonist 2-chloroadenosine (2-CA, 2μM) was 41±7% (n=3). In contrast, 5'-N-ethylcarboxamidoadenosine (NECA, 2μM) which is a relatively selective $A_2$ agonist, slightly enhanced glutamate release by 22±17% (n=10). It is thus likely that 8-PT potentiates glutamate release by blocking the effect of endogenous adenosine on $A_1$ receptors.

To investigate further the ability of 8-PT to enhance transmitter release, its effect was examined on spontaneous activity recorded intracellularly from CA1 and CA3 pyramidal cells in organotypic cultures where the synaptic organization of the hippocampus is maintained. Bath application of 0.1-1μM 8-PT reversibly increased both the number and size of spontaneous e.p.s.p.s, and caused bursting activity in some cells. This effect was blocked by the glutamate antagonist γ-D-glutamylglycine (1mM) but not by atropine (10μM) or bicuculline (100μM). Another adenosine antagonist isobutylmethylxanthine (IBMX, 10μM) had a similar effect to 8-PT, and its ability to enhance spontaneous e.p.s.p.s was blocked by 2-CA (20μM). In the absence of IBMX, 0.2μM 2-CA was sufficient to prevent spontaneous activity in these cells. At the concentrations used, IBMX and 8-PT had no effect on the resting membrane potential or input resistance of the cells. This suggests that their effect on spontaneous e.p.s.p.s has a presynaptic origin.

In conclusion, the present results suggest that endogenous adenosine inhibits both spontaneous release of excitatory transmitter from hippocampal organotypic cultures, and also $K^+$-stimulated glutamate release from dissociated cultures of cerebellar neurones. The source of the endogenous adenosine in these two systems remains to be identified.

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# SIMPLE MEASUREMENT OF AMINO ACID-INDUCED POLARIZATION OF HIPPOCAMPAL CA1 PYRAMIDAL NEURONES IN VITRO.

J.F. Blake*, M.W. Brown, G.L. Collingridge[1] and R.H. Evans[1], Departments of Anatomy and [1]Pharmacology, Medical School, University Walk, Bristol, BS8 1TD.

A simple in vitro method developed originally for quantitative studies of neuroactive compounds on spinal neurones (Curtis et al 1961) has been adapted more recently for measurements from cuneate and neocortical neurones (Simmonds 1978, Harrison & Simmonds 1985, Wheatley, 1986). The present paper describes the application of this method to agonist-induced changes in polarity of hippocampal CA1 pyramidal neurones.

The alveus, which contains axons of CA1 pyramidal neurones, was separated from 400 μm thick transverse hippocampal slices (prepared in the usual way from rat brains) leaving a connection to the CA1 region. This was achieved with a scalpel cut which extended from the subicular end of the slice to approximately the mid point of the CA1 region. The preparation was placed between layers of absorbent paper supported on an inclined heated block (25°C). Gassed (95/5% $O_2/CO_2$) $Mg^{2+}$-free bathing medium (mM: NaCl 124, $NaH_2FO_4$ 1.25, $NaHCO_3$ 26, KCL 5, $CaCl_2$ 2, D-glucose, 10) was superfused at 1.5 ml per min. The distal end of the fibre tract was raised onto a non-polarizable wick electrode and a grease barrier was placed around it to prevent dessication. DC polarity was recorded between this electrode and a similar one in the bathing medium.

In five preparations from different animals application of the excitant amino acids kainate, N-methyl-D-aspartate (NMDA) or quisqualate, (2-50 μM) for 1-2 min, evoked dose dependent depolarizations of up to 0.5 mV. These were not reduced in the presence of sufficient tetrodotoxin to block regenerative activity. In three preparations mean equimolar potency ratios ± s.e. of mean, determined at concentrations up to 5 times that required to elicit a threshold response, for kainate and quisqualate (relative to NMDA=1) were 2.6±0.4 and 3.5±0.6 respectively.

The greater depolarizing potency of NMDA relative to kainate and quisqualate, in this preparation compared to spinal cord and cerebral cortex (EVans 1978, Wheatley 1986), is consistent with the high density of NMDA binding sites present on CA1 neurones (Monaghan et al, 1984). This new preparation should prove useful for the quantitative analysis of excitant amino acid action, excitatory synaptic transmission, plasticity and epileptiform activity in the hippocampus.

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# TOLERANCE BETWEEN THE ANTINOCICEPTIVE EFFECTS OF MORPHINE AND STIMULATION OF THE ANTERIOR PRETECTAL NUCLEUS

Rawlings, S.R., Rees, H.* and Roberts, M.H.T. Department of Physiology, University College Cardiff.

Studies on forebrain stimulation in the rat revealed antinociception and aversion to be highly correlated (Prado & Roberts, 1985), in particular electrical stimulation of both periaqueductal gray (PAG) and nucleus raphe magnus were associated with strong escape reactions. However Prado & Roberts (1985) demonstrated a site, the pretectal nucleus (Pt.N) on the dorso-medial diencephalic border, where brief (15 sec) and mild (35 μA r.m.s.) electrical stimulation caused potent antinociception lasting 40 - 60 min. which was not associated with aversive behavioural responses. Recent studies have demonstrated that the antinociceptive effects of stimulating the Pt.N. are longer lasting than the antinociceptive effects of stimulating the PAG and less disrupting to motor performance (Roberts & Rees, 1986). The present study examines the likely involvement of opioid mechanisms in antinociception caused by electrical stimulation of the Pt.N.

The antinociceptive effects of morphine (5 mg/kg s.c., 16 animals) or Pt.N. stimulation (35μA for 15 sec., 10 animals) were assessed with the tail flick escape from noxious heat. Following these tests the animals were treated daily with doses of morphine or saline s.c. for one week and the effects of morphine or pretectal stimulation tested again. The first test dose of morphine caused a long lasting increase in tail-flick latency. The daily treatments with saline did not alter the antinociceptive effects of a second test dose of morphine. However, daily treatment with morphine severely attenuated the effects of the second test dose, demonstrating the development of tolerance to morphine. Electrical stimulation of the Pt.N. also caused a long lasting increase in tail-flick latency but following the daily treatments with morphine the antinociceptive effects of Pt.N. stimulation were severely attenuated. These observations indicate that animals that have developed tolerance to morphine also show attenuated responses to pretectal stimulation suggesting that opioid mechanisms are involved. To confirm this possibility 5 animals were pretreated with 1 mg/kg i.p. naloxone. This treatment also severely attenuated the effects of pretectal stimulation.

We conclude from these results that in common with many other antinociceptive stimulation sites in the brain, Pt.N stimulation activates opioid mechanisms.

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# ICI 174864 ANTAGONISES [MET]ENKEPHALIN-INDUCED DEPRESSION OF CHEMOSENSORY DISCHARGE IN ANAESTHETIZED CATS.

D.S. McQueen, Department of Pharmacology, University of Edinburgh Medical School, Edinburgh, EH8 9JZ.

[Met]enkephalin-like material is present in the cat carotid body (Wharton et al, 1980). Intra-carotid (i.c.) injection of [Met]enkephalin depresses chemosensory discharge, and this effect is antagonised by naloxone (McQueen & Ribeiro, 1980). The selective δ opioid receptor antagonist ICI 174864 (Cotton et al, 1984) was used in the present study to help characterise the type of opioid receptor involved in enkephalin-induced depression of chemosensory discharge.

Experiments were performed on cats anaesthetized with pentobarbitone (42mg $kg^{-1}$ i.p.), artificially ventilated with air, and paralysed with gallamine (3mg $kg^{-1}$ i.v.) Chemosensory discharge was recorded from the peripheral end of a sectioned carotid sinus nerve using techniques described previously (e.g. McQueen & Ribeiro, 1980). Drugs were injected into the common carotid artery close-arterial to the carotid body from which chemoreceptor activity was recorded, and the averaged discharge during the 30s period immediately following an injection was expressed as a percentage change from the pre-injection frequency. The $ID_{50}$ (dose causing a 50% reduction in discharge) was calculated for [Met]enkephalin from dose-response lines using data obtained before and after administering the opioid antagonist ICI 174864.

The mean $ID_{50}$ (± s.e. mean) for [Met]enkephalin-induced chemodepression was 0.51 ± 0.17 nmoles (n = 5) before the antagonist; values after 0.1 and 0.2mg $kg^{-1}$ i.c. of ICI 174864 were 4.05 ± 1.5 (n = 4) and 150 ± 81 (n = 5) nmoles respectively. ICI 174864 was 10-20 times more potent than ICI 154129 (Kirby & McQueen, 1986) in competitively antagonising chemodepression induced by [Met]enkephalin.

The results provide further evidence for involvement of δ type opioid receptors in opioid-induced depression of discharge from cat carotid body arterial chemoreceptors in vivo.

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# ARE THERE MULTIPLE OPIOID RECEPTOR TYPES IN THE RAT VAS DEFERENS?

A.G. Hayes and KM.J. Sheehan,
Department of Neuropharmacology, Glaxo Group Research, Ware, Herts.

It is generally agreed that the rat vas deferens possesses μ-opioid receptors but some workers suggest that it also contains either ε-receptors selective for β-endorphin (Schulz et al., 1979) or δ-opioid receptors (Smith and Rance, 1983). We have investigated further the nature of the opioid receptors in this tissue.

Isolated vasa deferentia from PVG rats were incubated in Krebs bicarbonate medium of yarying calcium concentration. In 2.5mM $Ca^{2+}$, the peptides β-endorphin, [D-$Ala^2$, $MePhe^4$, $Gly(ol)^5$] enkephalin (DAGO) and [D-$Ala^2$, D-$Leu^5$] enkephalin (DADLE) were able to completely inhibit the electrically-evoked twitches of the tissue (Table 1), while normorphine was a partial agonist and morphine had no agonist effect (although it antagonised DAGO). Reducing [$Ca^{2+}$] to 1.25 or 0.63mM progressively increased the potency of the full agonists, enabled normorphine to completely inhibit the twitches, and revealed a partial agonist action of morphine, suggesting that varying [$Ca^{2+}$] can alter the apparent receptor-effector coupling in the tissue.

Table 1 Agonist $IC_{50}$ values and antagonism by naloxone

| Drug | $IC_{50}$ (M) | | Naloxone $pA_2$ (slope) |
|---|---|---|---|
| | 2.5mM $Ca^{2+}$ | Low $Ca^{2+}$* | |
| β-endorphin | $4.5 \times 10^{-7}$ | $2.4 \times 10^{-8}$ | 8.3 ($0.85^x$) |
| DAGO | $3.8 \times 10^{-6}$ | $6.6 \times 10^{-7}$ | 8.6 (1.05) |
| DADLE | $6.1 \times 10^{-6}$ | $7.0 \times 10^{-7}$ | 8.5 (1.00) |
| normorphine | Emax 15% at $10^{-4}$M | $10^{-5}$ | 8.6 (0.92) |
| morphine | Antagonist | Emax 15-40% at $10^{-5}$M | 8.4 (assumed 1) |

* 1.25mM except for morphine (0.63mM). $^x$Significantly different from 1.

The naloxone $pA_2$ values for all compounds except β-endorphin were consistent with an action at μ-opioid receptors. The value against β-endorphin was significantly lower ($p<0.05$) than that for DAGO, and an even larger difference was observed in the $pA_2$ values for antagonism by Mr 2266 (8.7, slope 1.1, for DAGO and 7.9, slope 0.95, for β-endorphin). If β-endorphin acted partly at δ-opioid receptors, the apparent $pA_2$ value might tend towards the observed values. However, the δ-antagonist ICI 174864 did not antagonise DADLE or β-endorphin even with naltrexone present. PVG rats thus differ from Sprague-Dawley rats (Smith and Rance, 1983) in that they lack δ-opioid receptors.

Pretreatment of the preparation with the irreversible opioid antagonist β-chlornaltrexamine ($10^{-8}$M, 15min) antagonised the responses to DAGO and β-endorphin. Co-incubation with DAGO ($3 \times 10^{-5}$M) or Mr 2266 ($10^{-8}$M) failed to produce any differential protection of the response to DAGO or β-endorphin. This result does not support the existence of separate receptor types. We are currently investigating possible reasons for these discrepant data.

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# METHODOLOGICAL ASPECTS OF THE RECORDING OF MONOPHASIC ACTION POTENTIALS FROM THE PERFUSED GUINEA PIG HEART

O. Almgren, G. Duker and J. Reid* (Introduced by B. Åblad), Department of Pharmacology and Biochemistry, Hässle Cardiovascular Research Laboratories, S-431 83 Mölndal, Sweden.

The recording of monophasic action potentials (MAPs) has been shown to be an acceptable method to study repolarization changes in the in vivo guinea-pig heart (Duker et al., 1983). However, it is not possible to measure changes produced by known concentrations of drugs and ions in vivo. Therefore, we have developed and evaluated an in vitro methodology for this purpose.

Guinea-pig hearts were superfused horizontally at constant pressure (60 cm $H_2O$) and temperature (37°C) with Krebs-Henseleit buffer containing 9 mM glucose and 2 mM pyruvate as substrates. Epicardial MAPs were obtained using a bipolar Ag-AgCl electrode to which suction was applied. MAP amplitude, rise time and duration at 50, 75 and 90% repolarization levels (MAPD 50, 75 and 90) were determined by computer analysis (Duker et al., 1983). Electrograms were also recorded. Hearts were paced via the right atrium.

MAP signals had a smooth repolarization phase with amplitudes of 20-50 mV. A range of suction pressures (50-600 mmHg) was applied to the MAP electrode (n=6) so that a suitable pressure could be selected for routine use; 400 mmHg was chosen on the basis of maximal amplitude and stability of MAPD with time. In a second study, the right ventricular base was selected as the most appropriate region for the registration of MAPs because (a) amplitude was highest (34.8±1.4 mV, n=6), (b) MAPD was most reproducible over a 120-min period (MAPD 50, 75 and 90: 85.8±0.6, 102.5±0.5 and 111.9±0.5 ms, respectively; n=6), and (c) access to this region was easiest with the MAP electrode. There was no significant correlation between amplitude and rise time or between amplitude and MAPD ($p>0.05$, n=6). However, a significant correlation was found between heart rate and MAPD ($0.01>p>0.001$, n=3), illustrating the importance of pacing the heart at a constant rate. In some experiments, it was shown that the sinus node region could be cut away, resulting in the lowering of basal heart rate from approximately 240 to 160 beats/min. The removal had no effect on MAPD 50, 75 or 90, or on rise time ($p>0.05$, n=8), but caused a small significant rise in flow rate from 4.69±0.17 to 4.89±0.13 ml $min^{-1}$ $g^{-1}$ ($0.05>p>0.01$, n=6, atrial pace 270 beats/min).

The sensitivity of the method to detect changes in MAPD was examined using clofilium, a compound known to homogenously prolong single cell action potential duration, and tocainide, which decreases duration and increases rise time of the single cell action potential. Hearts were perfused with drug for 30 min after an initial 30-min perfusion in drug-free buffer. Clofilium ($10^{-8}$M to $10^{-5}$M) significantly prolonged MAPD 50, 75 and 90 in a dose-dependent manner with a maximal increase at $10^{-6}$M of 24, 26 and 22%, respectively. Tocainide ($3x10^{-4}$M) significantly decreased MAPD 50, 75 and 90 by 10, 8 and 6%, respectively ($0.01>p>0.001$, n=6) and increased rise time from 4.19±0.19 to 11.91±0.88 ms ($p<0.001$, n=6).

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# CARDIOVASCULAR ACTIONS OF 5'-N-ETHYLCARBOXAMIDEADENOSINE (NECA) AND L-$N^6$-PHENYLISOPROPYLADENOSINE (L-PIA) IN THE ANAESTHETISED RAT

P. Bhalla*, M.F. Gurden & I. Kennedy.

Department of Respiratory Pharmacology & Biochemistry, Glaxo Group Research, Ware, Herts, SG12 0DJ.

Adenosine and its analogues produce hypotension in anaesthetised animals (Angus et al., 1971; Phillis, 1982). These agents depress myocardial function and relax vascular smooth muscle (Collis, 1983; Collis & Brown, 1983), and either or both of these effects could result in hypotension. We have therefore examined the cardiovascular actions of two adenosine analogues, 5'-N-ethylcarboxamideadenosine (NECA) and L-$N^6$-phenylisopropyladenosine (L-PIA), in the anaesthetised rat in an attempt to determine the extent of the myocardial and vascular contribution to the hypotensive effects of these compounds.

Male AH/A strain rats (250-350g) were anaesthetised with sodium thiobutobarbitone (130-150mg/kg i.p.). The trachea was intubated and the animals allowed to breathe spontaneously. The left carotid artery and left femoral vein were cannulated for measurement of systemic blood pressure (BP) and administration of drugs respectively. Heart rate was derived from an ECG signal (Lead II). An electromagnetic flow probe was placed around the abdominal aorta, immediately below the diaphragm. Vascular resistance (VR) was calculated from mean BP and aortic flow (AF), either on line using a microcomputer or manually. Dose-effect curves to the agonists were constructed cumulatively, with only one agonist being tested in each animal. $ED_{50}$ values were calculated as mean $\pm$ S.E.M.

Both NECA and L-PIA ($10^{-11}$ - $10^{-6}$ mol/kg; n=5) caused dose-dependent falls in BP, HR and AF. These effects were rapid in onset (<1.5 min), but recovery was not seen for up to 3 hr. NECA ($ED_{50}$: $1.0\pm0.5\times10^{-9}$ mol/kg) was nine times more potent than L-PIA ($ED_{50}$: $9.0\pm1.2\times10^{-9}$ mol/kg) in reducing mean BP. The analogues were approximately equipotent in reducing HR ($ED_{50}$: $2.9\pm1.2\times10^{-9}$ and $7.0\pm1.2\times10^{-9}$ mol/kg, respectively) and AF ($ED_{50}$: $3.7\pm1.4\times10^{-9}$ and $11\pm1.3\times10^{-9}$ mol/kg, respectively). At doses up to $10^{-8}$ mol/kg, neither compound affected stroke volume, although this was decreased at high doses ( $10^{-6}$ mol/kg). However, whilst low doses of NECA (up to $10^{-8}$ mol/kg) caused dose-related decreases in VR, over the same dose range, L-PIA was without effect. Furthermore, at doses up to $10^{-6}$ mol/kg some reversal of the decrease in VR was observed with NECA, but L-PIA induced an increase in VR.

The adenosine receptor blocking drug, 8-phenyltheophylline (Griffith et al., 1981), given intravenously 5 min before, antagonised the effects of both agonists on all cardiovascular parameters, resulting in dose ratios of 50-80 at $10^{-5}$ mol/kg.

In conclusion, both NECA and L-PIA reduce BP in the anaesthetised rat. However, whilst NECA appears to do so by an effect on both the heart and the vasculature, L-PIA appears to act specifically on the heart.

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# EFFECTS OF $\alpha_2$-ADRENOCEPTOR ANTAGONISTS ON PLASMA CATECHOLAMINES AND ISCHAEMIA-INDUCED ARRHYTHMIAS IN THE ANAESTHETISED RAT

M. Avkiran*& B. Woodward, Pharmacology Group, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY.

Phentolamine suppresses ischaemia-induced arrhythmias (Sheridan et al, 1980). This effect could be due to $\alpha_1$ and/or $\alpha_2$ antagonism or some other non-specific action. We have investigated the effects of the $\alpha_2$-antagonists yohimbine (0.1 mg/kg i.v.) and idazoxan (0.3 mg/kg i.v.) on coronary ligation induced arrhythmias and plasma catecholamines in the anaesthetised rat, both in the absence and presence of the uptake-1 blocker desipramine (0.1 mg/kg i.v.). The experimental protocol was as previously described (Avkiran & Woodward, 1985).

Table 1 Effects of yohimbine(YOH), idazoxan(IDA) and desipramine(DMI) on blood pressure(BP), heart rate(HR) and arrhythmias

| Drug | n | Δ BP (mm Hg) | Δ HR (beats/min) | Total PVC | Incid. VT(%) | Incid. VF(%) | Deaths (%) |
|---|---|---|---|---|---|---|---|
| Saline | 52 | + 1(1) | + 4(2) | 732 (111) | 87 | 27 | 8 |
| YOH | 18 | + 1(2) | + 4(3) | 960 (198) | 94 | 56* | 33* |
| YOH/DMI | 14 | +25(4)* | + 83(5)* | 629 (171) | 79 | 21 | 7 |
| IDA | 18 | +30(4)* | + 36(7)* | 796 (168) | 83 | 56* | 22 |
| IDA/DMI | 14 | +60(6)* | +114(9)* | 361 ( 96)* | 71 | 36 | 0 |

Data expressed as mean (s.e.mean) *P<0.05 (v saline) PVC-premature ventricular contractions; VT-ventricular tachycardia; VF-ventricular fibrillation

YOH alone had no significant effect on plasma catecholamines (CA). However, the plasma noradrenaline (NA) concentration was significantly higher than controls (4.3±0.6 pmol/ml, n=6) in animals treated with YOH/DMI (6.4±0.7), IDA (8.4±0.6) and IDA/DMI (11.6±2.1). Plasma adrenaline was not affected. The elevated NA levels were reflected by significant increases in BP and HR (Table 1). Both $\alpha_2$-antagonists caused a significant rise in the incidence of VF and tended to increase mortality despite their differing effects on plasma NA, BP and HR. Concomitant administration of DMI abolished the arrhythmogenic effects of YOH and IDA (Table 1).

These results suggest that, unlike phentolamine, YOH and IDA exacerbate the arrhythmias induced by coronary occlusion. The mechanism appears to be unrelated to changes in plasma CA and haemodynamic alterations, although a possible effect on the local release of NA in the ischaemic myocardium cannot be excluded. The protective effect of phentolamine is therefore likely to be due to $\alpha_1$-antagonism or a class I antiarrhythmic action (Northover, 1983), and not $\alpha_2$-antagonism. DMI may abolish the arrhythmogenic effects of YOH and IDA by its inherent ability to block fast sodium channels (Tamargo et al, 1979) or by inhibiting a local efflux of NA in the ischaemic myocardium (Schömig et al, 1984), although it is not antiarrhythmic on its own at that dose (Avkiran & Woodward, 1985).

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280P

# DIFFERENTIAL EFFECT ON DILAZEP ON ADENOSINE METABOLISM IN THE GUINEA-PIG AND RAT HEART

Parviz Meghji* Kate Middleton & Andrew C.Newby (introduced by J.A. Smith)
Department of Cardiology, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN.

Nucleoside transport inhibitors such as dipyridamole, hexobendine and dilazep potentiate the inhibitory effects of adenosine in the guinea-pig heart (Fujita et al.,1980) by inhibiting adenosine uptake and increasing its concentration at extracellular receptor sites. These drugs are less potent in the rat than in other species (Williams et al., 1984), but even at concentrations that inhibit adenosine transport they do not potentiate adenosine effects (Burnstock & Meghji, 1983; Williams et al.,1984). Since an exo-form of adenosine deaminase has been demonstrated in some tissues (Andy & Kornfeld, 1982) a possible explanation of these findings is that adenosine is inactivated in the rat heart by extracellular deamination rather than intracellular metabolism.

Isolated hearts were paced (5Hz,5V) and perfused at 10 ml/min with oxygenated (95% $O_2$/5% $CO_2$) Krebs-Henseleit solution at 37°C. After 30 min of equilibration buffer with or without dilazep was infused at 1 ml/min for 30 min and a sample of effluent was collected. The infusion was then supplemented with adenosine (11 μM) and [$^3$H]-adenosine (5 KBq/ml) for 10 min and a second sample of effluent was collected. The infusion was then stopped, perfusion continued for 5 min and the hearts were then freeze-clamped, extracted and the radioactivity incorporated into nucleotides determined (Newby et al., 1983). The concentration of metabolites released into the perfusate was measured by h.p.l.c. (Newby et al., 1983) and the difference before and during adenosine infusion was computed.

| | Recovery of infused adenosine (%) | | | |
|---|---|---|---|---|
| Guinea-Pig | Effluent Adenosine | Effluent Inosine | Effluent Uric Acid | Cellular Nucleotides |
| Control (n=9) | 73.9 ± 3.5 | 6.5 ± 0.9 | 7.6 ± 2.4 | 7.9 ± 0.2 (n=5) |
| Dilazep 0.05 μM (Final concn.,n=6) | 99.3 ± 5.7*** | 0.7 ± 0.6*** | -1.1 ± 2.6* | 1.0 ± 0.1(n=9)*** |
| Rat | | | | |
| Control (n=10) | 9.7 ± 2.2 | 16.9 ± 2.8 | 51.8 ± 5.2 | 7.0 ± 0.2 (n=5) |
| Dilazep 10 μM (Final concn.,n=8) | 17.0 ± 1.8* | 16.4 ± 1.4 | 34.2 ± 2.1* | 2.3 ± 0.2(n=8)*** |

* $P < 0.05$, ** $P < 0.01$, ***$P < 0.001$ compared to control

In both species inosine and uric acid were the major catabolites of adenosine recovered while 7-8% was incorporated into cellular nucleotides. In the guinea-pig heart, incorporation of adenosine into nucleotides,a measure of nucleoside transport and catabolism to inosine and uric acid were both inhibited by > 90% by dilazep (0.05 μM). In the rat heart, the metabolism of adenosine decreased by 8% and the recovery of inosine and uric acid decreased by 26% in the presence of dilazep (10 μM) which inhibited adenosine incorporation by 70%. This suggests that only 10-40% of the adenosine metabolism is intracellular and thus that extracellular deamination is the main mechanism of adenosine inactivation. These observations may account for the difference in the effect of nucleoside transport inhibitors on responses to adenosine in the two species.

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# PRESYNAPTIC EFFECT OF ADRENALINE ON STIMULATION-EVOKED [$^3$H]-NORADRENALINE RELEASE FROM RABBIT ISOLATED AORTA.

J. Abrahamsen* & O.A. Nedergaard, Department of Pharmacology, School of Medicine, Odense University, J.B. Winsløws Vej 19, DK-5000 Odense C, Denmark.

It is possible that adrenaline is implicated in the development of certain types of hypertension. Furthermore, it has been suggested that adrenaline can enhance depolarization-evoked noradrenaline release from sympathetic nerves through activation of facilitatory presynaptic β-adrenoceptors (Majewski & Rand, 1981; 1984; Majewski 1983). [$^3$H]-adrenaline can be taken up by rabbit aorta (Abrahamsen & Nedergaard 1985) from where it can be released by electrical-field stimulation (Abrahamsen & Nedergaard 1986). In the present work we studied the effect of (-)-adrenaline on (-)-[$^3$H]-noradrenaline ([$^3$H]-NA) release evoked by field stimulation of rabbit isolated aorta. The method described previously (Nedergaard 1980) was used.

Adrenaline ($10^{-10}$-$3x10^{-9}$M) had no effect on the stimulation-evoked [$^3$H]-overflow from aorta preloaded with [$^3$H]-NA; at $10^{-8}$ and $3x10^{-8}$M the [$^3$H]-overflow was decreased by up to 47%. Cocaine ($3x10^{-5}$M) + corticosterone ($4x10^{-5}$M) augmented the inhibitory effect of adrenaline ($10^{-9}$-$10^{-6}$M). The $IC_{50}$ was $2x10^{-8}$M and the maximal decrease was 85%. In the presence of either normetanephrine ($4x10^{-5}$M) + cocaine ($3.3x10^{-6}$M) or normetanephrine ($10^{-5}$M) + desmethylimipramine ($10^{-6}$M), similar concentration-response curves were obtained with adrenaline ($10^{-10}$-$10^{-6}$M).

With cocaine + corticosterone present, the adrenaline-induced inhibition of stimulation-evoked [$^3$H]-overflow was dependent on the frequency (1, 3 or 8 Hz): The response curve obtained at 1 Hz was successively moved to the right at 3 and 8 Hz. Adrenaline ($10^{-9}$ and $3x10^{-7}$M) in a concentration-dependent manner only reduced the [$^3$H]-overflow evoked by stimulation at various frequencies (1, 2, 4, 8 and 16 Hz). The degree of inhibition varied slightly in a complex manner with the frequency: Adrenaline ($3x10^{-7}$M) inhibited maximally at 4 Hz and less so at the other employed frequencies. Just the converse was seen with $10^{-9}$M adrenaline.

We studied the influence of α-adrenoceptor blockade on stimulation-evoked [$^3$H]-overflow from aorta preloaded with [$^3$H]-NA. Cocaine + corticosterone were present. Rauwolscine ($10^{-6}$M) and phentolamine ($10^{-6}$M) moved the concentration-response curve for the inhibitory effect of adrenaline ($10^{-9}$-$10^{-6}$M) to the right. In the presence of phenoxybenzamine ($10^{-6}$M), adrenaline ($10^{-9}$ - $10^{-6}$M) enhanced by up to 31% the stimulation-evoked [$^3$H]-overflow. Neither (±)-propranolol ($10^{-6}$M) nor metoprolol ($10^{-6}$M) antagonized the enhancing effect of adrenaline, while atenolol ($10^{-6}$M) did for $10^{-7}$M of adrenaline.

We conclude that sympathetic nerves in the rabbit aorta possess presynaptic inhibitory $\alpha_2$-adrenoceptors, but not facilitatory β-adrenoceptors.

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# TOLERANCE TO NITROGLYCERIN IN ISOLATED RABBIT AORTA DOES NOT ALTER ENDOTHELIUM DEPENDENT RELAXATION TO ACETYLCHOLINE OR A23187

A. Hughes, G. Martin*, M. Schachter, P. Sever. Dept. of Clinical Pharmacology, St. Mary's Hospital Medical School, Norfolk Place, London, W.2.

The endothelium derived relaxing factor (EDRF) has received a great deal of attention since its discovery in rabbit aorta by Furchgott and Zawadzki (1980). However, the chemical nature of EDRF remains unknown. EDRF has been likened to an "endogenous nitrate" since, like nitrates, its vasodilatory mechanism is linked to a rise in intracellular cGMP in smooth muscle (Peach et al., 1985). However, it is not clear whether the activation of guanylate cyclase occurs by a similar mechanism in both cases. Therefore we have investigated the effect of nitroglycerin (NG) tolerance in the classical model system for EDRF, the in vitro rabbit aorta.

Seven New Zealand white rabbits were killed by a blow to the head. The descending thoracic aortas were removed and mounted as rings in tissue baths under a resting tension of 1g. The baths contained 6ml Krebs solution aerated with 95% $O_2$/5% $CO_2$ and maintained at 37°C throughout. The aortic rings were preconstricted with noradrenaline (1μM) (approx. EC50), followed by the preparation of cumulative dose response curves to acetylcholine (10nM-100μM), the calcium ionophore A23187 (30nM-1μM), NG (10nM-100μM) and sodium nitroprusside (100nM-10μM). The rings were incubated with NG ($5.5 \times 10^{-4}$M) for 60 mins (pH 7.4), washed out, and the cumulative dose response curves repeated after reconstriction. The rings were then incubated with dithiothreitol (0.5mM) for 30 mins (pH 7.4), washed out, and the dose response curve for NG repeated again. Relaxation was calculated as percent reduction of preconstricted tone.

NG produced a dose dependent relaxation (EC50 0.12μM, maximum 74%). Following incubation with NG there was no relaxation to NG over the concentration range tested. There was no detectable cross tolerance to sodium nitroprusside. Tolerance to NG was only partially reversed by treatment with dithiothreitol (EC50 5.3μM, maximum 67%).

Incubation with NG did not significantly influence endothelium dependent relaxation to either acetylcholine or A23187. Acetylcholine produced a dose dependent relaxation both before (EC50 0.5μM, maximum 64%) and after induction of tolerance (EC50 0.3μM, maximum 64%). Similarly A23187 produced similar dose dependent relaxation before (EC50 0.3μM, maximum 63%) and after induction of tolerance (EC50 0.3μM, maximum 52%).

These findings indicate that the action of EDRF in response to either acetylcholine or A23187 is unaffected by inhibition of the mechanism mediating vasodilatation to NG. Therefore the availability of nitrosothiol intermediates required for vascular relaxation to NG (Needleman et al., 1973) does not appear to be crucial for EDRF mediated relaxation.

It has been suggested that there may be species differences in the nature of EDRF (Forstermann et al., 1984). However, similar results for acetylcholine induced relaxation have recently been reported using isolated bovine intrapulmonary artery (Lieberman et al., 1985).

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# ALTERATIONS IN CARDIAC β-ADRENOCEPTOR NUMBER AFTER REVERSIBLE NORADRENERGIC DENERVATION

T. Bennett[+], P.G. Strange and K. Williams*, Departments of Biochemistry and Physiology and Pharmacology[+], University of Nottingham Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

Several authors have reported an increase in the number of cardiac β-adrenoceptors after noradrenergic denervation with 6-hydroxydopamine (6OHDA). In the chick heart, 6OHDA produces a reversible noradrenergic denervation (Bennett and Malmfors, 1974). We have examined changes in β-adrenoceptor number of chick left atrium after 6OHDA-induced denervation and during the subsequent re-innervation period. Chicks (22-25 days old) were injected i.v. with either 6OHDA (100mg/kg in 0.9% NaCl + 0.1% acorbic acid) or with vehicle only (controls). At various times after injection chicks were killed, the left atrium removed and homogenized in HEPES-buffered physiological medium (Bennett et al., 1985). Aliquots (300-500μg protein) of atrium homogenate were incubated with [$^3$H]-dihydroalprenolol (DHA, 0.8nM final) for 30 minutes at 25°C. Non-specific binding was defined with 200μM (L)-isoprenaline and all incubation samples contained 0.1mM ascorbic acid. Bound and free radioligand were separated by filtration over Whatman GF/B filters. Tissue catecholamines were extracted by a modification of the method of Anton and Sayre (1962) and noradrenaline (NA) levels measured by HPLC with electrochemical detection. Table 1 shows specific [$^3$H]-DHA binding and NA content of the left atrium at various times after 6OHDA treatment.

Table 1

| Days after injection | Specific [$^3$H]-DHA bound (fmoles/mg protein, mean ± S.E. mean (n)) CONTROL | 6OHDA TREATED | 6OHDA-treated NA content % of control |
|---|---|---|---|
| 1 | 26.1 ± 2.3 (8) | 28.8 ± 1.9 (8) | 20.0 |
| 3 | 24.5 ± 1.5 (5) | 26.8 ± 1.6 (5) | ND |
| 5 | 27.9 ± 2.2 (5) | 35.0 ± 1.7 (5)* | ND |
| 7 | 25.5 ± 1.9 (7) | 36.0 ± 3.5 (7)* | 26.5 |
| 14 | 18.6 ± 1.3 (6) | 23.1 ± 1.5 (5) | ND |
| 21 | 19.0 ± 1.3 (6) | 16.6 ± 0.4 (6) | 55.0 |

* $p<0.05$ by Student's unpaired t-test compared to control. ND - not determined

Saturation binding isotherms indicate that the increase in [$^3$H]-DHA binding is due to an increased number of β-adrenoceptors with no change in affinity for [$^3$H]-DHA: Control, $B_{max}$ = 63.6 ± 6.5 fmoles/mg protein, $K_D$ = 0.96 ± 0.15 nM; 7 day post 6OHDA treatment, $B_{max}$ = 85.6 ± 6.1 fmoles/mg protein, $K_D$ = 0.96 ± 0.13nM (mean ± S.E. mean, n = 4).

After 6OHDA-induced denervation there was a slowly developing increase in the number of β-adrenoceptors, returing to control levels 14 to 21 days after 6OHDA treatment, during which time there is substantial re-innervation of the left atrium. These results suggest that noradrenergic denervation and re-innervation have opposing effects on β-adrenoceptor number in chick atrium.

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# CHRONIC AGONIST REGULATION OF $\beta$-ADRENOCEPTORS IN THE RABBIT

N.M. Deighton,* C.A. Hamilton, C.R. Jones, J.L. Reid, Department of Materia Medica, Stobhill General Hospital, Glasgow, G21 3UW.

Longterm administration of a hormone or agonist can lead to an early loss of responsiveness or desensitisation succeeded by a slower more gradual down regulation of receptor number (Scarpace & Abrass, 1982). We have investigated $\beta$ adrenoceptor regulation in tissues containing predominantly $\beta_1$ or $\beta_2$ subtypes.

Groups of male New Zealand white rabbits (2-3 kg, n=6-8) were used. Long term (10 day) infusions of adrenaline (ADR) (0.05 $\mu$ moles/kg/hr) with high affinity for $\beta_1$ and $\beta_2$ receptors were given into a femoral vein via an implanted osmotic mini-pump; control animals received similar infusions of vehicle (0.1% ascorbate in 0.9% saline). Blood samples were removed for catecholamine measurement at 4 times during the 10 day infusion and mean arterial pressure (MAP) and heart rate (HR) measured at intervals throughout the study period. On day 10 the animals were killed and blood and heart removed. [$^{125}$I] Iodocyanopindolol (ICYP) 10-150 pM binding to whole platelets, lymphocytes and cardiac membranes was determined for controls and treated animals (Jones et al, 1985). The equilibrium binding constant, $K_D$ (pM) and maximum number of binding sites, Bmax (fmoles/mg protein or $10^8$ platelets/ml) were estimated by Scatchard analysis.

Basal plasma ADR levels were 1.4 ± 1.5 and rose to 10.4 ± 5.7 nM after ten days ADR infusion. Noradrenaline levels were 1.5 ± 0.4 before and 1.9 ± 1.5 nM after ten days. There were no significant alterations in MAP and HR (84 ± 5, 210 ± 31, before and 80 ± 9 mmHg, 228 ± 27 beats/min) after ten days. There was a significant reduction in [$^{125}$I] ICYP binding site number in cardiac membranes without any decreases in lymphocytes or platelets as shown below.

[$^{125}$I] ICYP Binding mean ± SD (n > 6)

| | Heart | | Lymphocyte | | Platelet | |
|---|---|---|---|---|---|---|
| | Control | (ADR) | Control | (ADR) | Control | (ADR) |
| Bmax | 51± 17 | 22± 7 | 15± 7 | 16± 6 | 3.4 ± 2 | 2.6± 1.8 |
| $K_D$ | 47 ± 20 | 54 ± 32 | 32 ± 14 | 51 ± 17 | 26 ± 12 | 44 ± 30 |

The $K_D$ values were not significantly altered by ADR infusion in any tissue studied.

Thus, under our experimental conditions a decrease in [$^{125}$I] ICYP binding in heart but not platelet or lymphocyte was observed. In the rabbit, adrenoceptors in heart are almost exclusively $\beta_1$ while those on platelets and lymphocytes are $\beta_2$ (Jones et al, 1985). Our findings are consistent with work on rat phaeochromocytomata (Tsujimoto, et al, 1984) which revealed down regulation in tissues containing $\beta_1$ but not $\beta_2$ adrenoceptors. Agonist induced down regulation of $\beta_1$ and $\beta_2$ adrenoceptors does not always go in parallel. This may reflect differences in regulation of the receptor subtypes.

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# THE EFFECT OF CENTRAL ADMINISTRATION OF METHOXAMINE, PHENYLEPHRINE AND ST 587 ON BLOOD PRESSURE AND HEART RATE IN THE RAT

Vanessa Pierce* and N.B. Shepperson, Department of Pharmacology, Wyeth Research (UK) Ltd, Huntercombe Lane South, Taplow, Nr Maidenhead, Berks.

Hypotension evoked by clonidine has been attributed to the stimulation of central $\alpha_2$-adrenoceptors, but the role of central $\alpha_1$-adrenoceptors in cardiovascular control is controversial. Central administration of phenylephrine (PE) results in hypotension and bradycardia, which can be blocked by phenoxybenzamine (Bhargava et al, 1972). A recent report that the selective $\alpha_1$-adrenoceptor agonist ST 587 (De Jonge et al, 1981) did not evoke a change in blood pressure or heart rate (HR), led to the conclusion that central $\alpha_1$-adrenoceptors do not mediate effects on cardiovascular centres (Pichler & Kobinger, 1985). The effects of central administration of the selective $\alpha_1$-adrenoceptor agonist methoxamine, ME, (Kobinger & Pichler, 1982) on diastolic blood pressure (DBP) and HR have now been compared with those of PE and ST 587 in the anaesthetised rat.

Groups of 4 female Sprague Dawley rats (230-270g) were anaesthetised with pentobarbitone (50 mgkg$^{-1}$ i.p.). DBP was recorded from the femoral artery and HR derived from the pulse pressure. ME, PE or ST 587 (5 µg or 50 µg in 10 µl) was administered into a lateral cerebral ventricle (i.c.v.). DBP and HR were recorded 1, 15 and 30 min later. After i.c.v. injection of the agonists, the $\alpha_1$-adrenoceptor antagonists indoramin (Alps et al, 1970) or Wy 23925 (N-[1-(2-[indol-3-yl] ethyl) piperid-4-yl]pyridine-4-carboxamide, an analogue of indoramin thought not to cross the blood brain barrier) were administered cumulatively intravenously (10 µg kg$^{-1}$ - 10 mgkg$^{-1}$ i.v.) or centrally (75-300 µg i.c.v.). The selective $\alpha_2$-adrenoceptor antagonist Wy 26392 (Paciorek et al, 1984) was also administered i.v. (1-300 µg kg$^{-1}$).

ME and PE evoked significant ($p < 0.001$) dose related decreases in DBP and HR 30 min after i.c.v. injection. 50 µg of ME and PE induced falls in DBP of 53 ± 3 mm Hg and 40 ± 5 mm Hg and falls in HR of 115 ± 14 beats min$^{-1}$ and 112 ± 7 beats min$^{-1}$ respectively. The hypotension (but not the bradycardia) evoked by 50 µg i.c.v. methoxamine was reversed by i.v. indoramin (0.3-3 mgkg$^{-1}$) but not by i.v. administration of Wy 23925. In contrast, i.c.v. administration of either antagonist reversed the hypotension and bradycardia evoked by methoxamine. I.C.V. Wy 23925 also reversed the hypotension and bradycardia evoked by phenylephrine. A dose of 0.5 µg i.c.v. clonidine induced a hypotension and bradycardia of similar magnitude to that evoked by 50 µg i.c.v. ME. The selective $\alpha_2$-adrenoceptor antagonist Wy 26392 (1-300 µg kg$^{-1}$ i.v.) reversed the effects of clonidine but not those of ME.

In contrast to ME and PE, 50 µg i.c.v. ST 587 did not evoke hypotension or bradycardia after 30 min; 5 µg ST 587 induced only a small decrease in DBP of 9 ± 3 mm Hg.

In conclusion central administration of ME and PE evokes hypotension and bradycardia in the anaesthetised rat. These effects appear to be mediated via $\alpha_1$-adrenoceptors as they could be reversed by the $\alpha_1$-adrenoceptor antagonists indoramin and Wy 23925 but not by the $\alpha_2$-adrenoceptor blocker Wy 26392. In agreement with De Jonge et al, (1981) and Pichler and Kobinger (1985), ST 587 had little effect on DBP and HR in the present experiments.

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# EVIDENCE FOR THE MEDIATION OF THE $C_1$ PRESSOR RESPONSE BY $\beta_2$-ADRENOCEPTORS

K.R. Borkowski[2], C.A. Marsden[1], P. Marshall[2] & C. Routledge[1]*; [1]Department of Physiology Pharmacology, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH and [2]Bioscience II, Imperial Chemical Industries plc., Pharmaceuticals Division, Mereside, Alderley Park, Macclesfield, Cheshire SK10 4TG.

Cell groups in the rostral ventrolateral medulla (RVL) are involved in the tonic and reflex control of blood pressure (Ross et al., 1983). These cells innervate the hypothalamus and the spinal cord, areas implicated in blood pressure regulation (Thornton et al., 1984; Ross et al., 1984) and evidence suggests they are adrenergic. Stimulation of the RVL has been shown to increase the release of adrenaline in the posterior hypothalamus (Routledge & Marsden, 1984). The present study shows that hypothalamic and spinal adrenoceptors may be involved in the pressor response during $C_1$ stimulation.

Male Alderley Park Wistar rats (270-300 g) were anaesthetised with Saffan (Glaxo, 18 mg/kg i.v. for induction and 0.3-1.4 mg/kg/min i.v. for maintenance). The left common carotid artery was cannulated for measurement of blood pressure. A modified SNE 100 (Clarke Electromedical Equipment) concentric needle electrode (diam. 100 μm) was stereotaxically implanted into the $C_1$ region of the RVL. The $C_1$ region was electrically stimulated (square wave pulses of 1 msec duration, 250 μA were passed at 10 Hz) for 10 s every 3 min. Following 3 control stimuli drugs were administered at different concentrations in artificial CSF (5 μl intra-cisternally, i.c. n=9; 1 μl intracerebroventricularly, i.c.v., n=9; 1 μl into the posterior hypothalamic area, i.p.h., n=6) and their effects on the pressor response to 3 further $C_1$ stimuli monitored 10, 15 and 20 min later. The positions of the electrodes were verified histologically.

$C_1$ stimulation caused a reproducible increase in mean arterial pressure of 28±5.6 mmHg. Administration of the β-adrenoceptor antagonist D,L-propranolol (17 μg i.c., i.c.v. and i.p.h.) decreased the pressor response by 14±0.4, 14±0.6 and 10±1.9 mmHg respectively ($P<0.05$). A smaller decrease was observed at a lower dose (8 μg). D,L-propranolol was without effect when given i.v. (17 μg) indicating that the effect was central. D-propranolol (17 μg) was without effect as was the specific $\beta_1$-adrenoceptor antagonist atenolol (33 μg). The $\beta_2$-adrenoceptor antagonist ICI 118551 (33 μg, i.c., i.c.v., and i.p.h.) decreased the pressor response by 15±1.0, 16±1.5 and 12±3.5 mmHg respectively ($P<0.05$). A lower dose (17 μg) also significantly decreased the pressor response. Idazoxan (66 μg) administered to each area enhanced the pressor response 11±1.3 i.c., 14±1.1 i.c.v. and 19±1.8 i.p.h. ($P<0.01$) with a smaller effect at a lower dose (33 μg), but had no effect when given i.v. (66 μg). C.S.F. administered to each area was without effect on the pressor response.

These results indicate a hypothalamic as well as a spinal involvement in the $C_1$ stimulated pressor response. The lack of effect of atenolol and the decrease following ICI 118551 administration suggest that the neural pathway mediating the pressor response involves central $\beta_2$-adrenoceptors with a possible regulatory role for $\alpha_2$-adrenoceptors.

CR is an SERC CASE student in conjunction with ICI plc.

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# [$^3$H]-BETAXOLOL IS RELEASED BY ELECTRICAL STIMULATION FROM RAT ATRIA BUT NOT VENTRICLE: EFFECTS OF DENERVATION WITH 6-OHDA

Sonia Arbilla*, S.Z. Langer, Palmina Petruzzo and Maria Vidal , Department of Biology, Laboratoires d'Etudes et de Recherches Synthelabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France.

We have recently demonstrated that the cardioselective β-adrenoceptor antagonist betaxolol (BTXL) can be accumulated and retained in rat atria to be subsequently released by electrical stimulation (Arbilla et al., 1986). The electrically-evoked release of $^3$H-BTXL was significantly reduced after stellate ganglionectomy or pretreatment with reserpine (Petruzzo et al., 1986), indicating that the presence of noradrenergic nerve endings and an intact storage mechanism was necessary to elicit the release of $^3$H-BTXL. The fact that tissue retention of $^3$H-BTXL was not affected by surgical ganglionectomy indicates that $^3$H-BTXL is not retained within the noradrenergic nerve terminal (Petruzzo et al., 1986). We have now evaluated the effects of 6-hydroxydopamine (6OHDA) on tissue accumulation and electrically-evoked release of $^3$H-BTXL or $^3$H-noradrenaline ($^3$H-NA) from atrial slices. Atrial and ventricle slices from normal male rats (Sprague-Dawley, 200-220 g, Charles River, France), or from rats pretreated with 6OHDA (100 mg/kg i.v., 48 h) were incubated for 30 min with 0.1 μM $^3$H-BTXL (S.A. 49 Ci/mmol) or for 15 min with $^3$H-NA (S.A. 9.4 Ci/mmol), and perfused in Krebs medium containing 2.6 mM $Ca^{2+}$ and 1 μM atropine. Electrical stimulation was performed in all cases at 5 Hz, 24 mA, 2 ms for 2 min. Endogenous levels of NA in atria were measured by HPLC chromatography with electrochemical detection.

Table 1 : Effects of 6OHDA on the release of $^3$H-BTXL and $^3$H-NA from rat atrium

| | $^3$H-BTXL | | | $^3$H-NA | | | Endogenous NA | |
|---|---|---|---|---|---|---|---|---|
| | $S_1$ (%) | T (a) | n | $S_1$ (%) | T (a) | n | (ng/g) | n |
| Control | 2.38 ± 0.31 | 32.0 ± 5.36 | 10 | 2.83 ± 0.5 | 59.4 ± 13.0 | 10 | 3863 ± 433 | 6 |
| 6OHDA | 0.51* ± 0.16 | 47.45 ± 8.29 | 9 | 0.30* ± 0.13 | 9.73* ± 1.0 | 8 | 166* ± 5.2 | 6 |

$S_1$ : fractional release of total radioactivity ; n = number of experiments per group ; shown are mean values ± S.E.M. ; (a) nCi retained per slice at the end of the experiment ; * $p < 0.05$ when compared with the corresponding value in control group.

In contrast with atrial slices, in ventricle slices, we were unable to elicit release of $^3$H-BTXL during electrical stimulation, while the release of $^3$H-NA was clearly present ($S_1$ = 0.88 ± 0.1 %, n = 12), although there was a significant tissue-retention of $^3$H-BTXL in the ventricle (42.81 ± 10.05 nCi/slice, n = 7). In atrial slices from rats pretreated with 6OHDA, the endogenous content of NA was significantly reduced compared to controls (Table 1), representing a decrease of 95.7 %, which was parallelled by an 80 % reduction in the retention of $^3$H-NA when compared to the controls. After 6OHDA, there was no decrease in the atrial retention of $^3$H-BTXL (Table 1). Pretreatment with 6OHDA reduced the release of $^3$H-NA by electrical stimulation by 89.4 % (Table 1). The release of $^3$H-BTXL by electrical stimulation was also significantly decreased by 78.6 % (Table 1). Our results show that $^3$H-BTXL can be accumulated in slices from rat atria or ventricle, but only in the atria, electrical stimulated evoked the release of $^3$H-BTXL. The fact that pretreatment with 6OHDA failed to reduce the tissue retention of $^3$H-BTXL indicates that $^3$H-BTXL is not retained in noradrenergic nerve terminals.

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# DOES KETANSERIN REDUCE ISCHAEMIA AND REPERFUSION-INDUCED ARRHYTHMIAS IN ANAESTHETISED RATS?

Susan J. Coker, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool, L69 3BX.

The 5-HT M receptor antagonist ICS 205-930 has been shown to have antiarrhythmic activity in anaesthetised rats and dogs subject to coronary artery occlusion and reperfusion (Williams et al., 1985; Coker et al., 1986) although the precise mechanism of this action has not been clarified. Ketanserin, an antagonist at 5-$HT_2$ receptors has also been reported to possess antiarrhythmic properties in vitro (Saman et al., 1985). The aim of this study was to determine whether ketanserin has antiarrhythmic activity in vivo and hence gain further information on the possible involvement of 5-HT in arrhythmogenesis.

Experiments were performed in male Wistar rats (260 to 330 g) which were anaesthetised with sodium pentobarbitone 60mg/kg i.p. Arterial blood pressure was measured from the left carotid artery and recorded along with a Lead I electrocardigram. A left thoracotomy was performed and a fine silk ligature was placed around the left coronary artery close to its origin. Arrhythmias induced by coronary artery occlusion were quantified as described by Clark et al, (1980). In separate groups of rats reperfusion arrhythmias were induced by releasing the ligature after 5 min of ischaemia according to the method of Kane et al, (1984).

The administration of ketanserin 1mg/kg i.v. 15 min prior to coronary artery occlusion caused substantial reductions in heart rate (408 $\pm$ 8 to 333 $\pm$ 19 beats/min, $P< 0.01$) and arterial blood pressure (116 $\pm$ 8/87 $\pm$ 8 to 91 $\pm$ 6/62 $\pm$ 5 mmHg, $P< 0.05$, t-test). Despite these marked haemodynamic effects of ketanserin the number of extrasystoles that occurred during the first 25 min of myocardial ischaemia (1492 $\pm$ 300) was not significantly different from that in controls (1402 $\pm$ 369). The durations of ventricular tachycardia (VT) and ventricular fibrillation (VF) were also similar in control and ketanserin treated rats; VT - 113 $\pm$ 27 and 116 $\pm$ 37 s respectively, VF - 103 $\pm$ 38 and 68 $\pm$ 25 s respectively. VT and VF occurred in 87% of the animals in each group and the mortality was 37% in both groups (n = 8).

Ketanserin also failed to reduce reperfusion-induced arrhythmias. VT occurred in 87% of control rats (n = 8), 83% of those which were pretreated with ketanserin 0.3 mg/kg i.v. 10 min prior to coronary artery occlusion (n = 6) and in 100% of the group which received 1 mg/kg ketanserin i.v. (n = 8). The corresponding values for the incidence of VF were 87%, 83% and 63% and the mortality was 75%, 67% and 63% in each group respectively.

These results suggest that if 5-HT is involved in the genesis of ischaemia or reperfusion-induced arrhythmias in anaesthetised rats, its effects do not depend on activation of 5-$HT_2$ receptors.

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# β-ADRENOCEPTOR ANTAGONIST ACTIVITY OF PROPAFENONE IN THE ANAESTHETIZED DOG.

N.L. Brown*, M. Worcel & J. Zanirato. Centre de Recherches Roussel-Uclaf, B.P. 9, 93230 Romainville, France.

The anti-arrhythmic drug propafenone, 2'-(2-hydroxy-3-(propylamino)propoxy)-3-phenyl-propiophenone, hydrochloride has been reported to possess non-selective β-adrenoceptor antagonist activity in vitro (McLeod et al., 1984). However there is no direct in vivo evidence of this non-selective β-antagonist activity and there is some doubt as to whether this effect will be observed at therapeutic anti-arrhythmic doses. The β-adrenoceptor blocking action of propafenone was therefore assessed in the anaesthetized ganglion-blocked dog at doses (1-4 mg/kg, i.v.) known to be anti-arrhythmic in this species (Karagueuzian et al., 1982 ; von Philipsborn et al., 1984). (±)-Propranolol was employed as a reference compound.

Male Beagle dogs, anaesthetized with sodium pentobarbital, were implanted with Millar pressure transducers so as to obtain carotid arterial pressure, left ventricular pressure and left ventricular dP/dt. Heart rate was determined by a cardiotachometer and the ECG was recorded. Dogs were ganglion-blocked with 1 mg/kg i.v. chlorisondamine and muscarinic receptors were blocked with 1 mg/kg i.v. methylatropine.Isoprenaline (0.1 ug/kg) was administered i.v. as a bolus injection every 20 min. Following two (control) isoprenaline administrations, animals received i.v. perfusion of either 5% dextrose (control group, n=6) or perfusions of (+)-propranolol.HCl (n=6) or (±)-propafenone.HCl (n=5) in 5% dextrose, such that further isoprenaline challenges were performed in the presence of 15, 30, 45 and 60 ug/kg propranolol or 1, 2, 3 and 4 mg/kg propafenone.

Analysis of variance revealed no significant difference amongst the 3 groups for baseline parameters and control responses to isoprenaline (increases in dP/dt, contractile index, heart rate and decreases in diastolic BP). In the control group responses to isoprenaline over the experimental period did not change significantly from the preinfusion values of increases of 3352±290 mmHg/sec for dP/dt, 44.5± 4.3 beats/min for heart rate, 27.7±3.7 $sec^{-1}$ for contractile index and a decrease of 36.2±3.7 mmHg for diastolic BP. Propafenone and propranolol produced significant and dose-dependent attenuation of the isoprenaline-induced responses (analysis of variance and Duncan's Multiple Range test), propafenone giving $ID_{50}$ of 1.86±0.62 mg/kg, 2.47±0.97 mg/kg, 1.95±0.20 mg/kg and 2.93±1.3 mg/kg for dP/dt, contractile index, heart rate and diastolic BP respectively. The corresponding values for propranolol were 29.42 ± 6.67 ug/kg, 36.62 ± 6.52 ug/kg, 38.66 ± 4.67 ug/kg and 42.16 ± 12.74 ug/kg giving a ratio of potencies for propranolol-propafenone of 1:63 (dP/dt), 1:67 (contractile index), 1:50 (heart rate) and 1:69 (diastolic BP).

These results confirm the non-selective β-adrenoceptor antagonist activity of propafenone observed in vitro and suggest that this activity will be present at anti-arrhythmic doses.

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# THE USE OF RAT AND GUINEA PIG CARDIAC ACTION POTENTIALS TO DISTINGUISH SUBCLASSES OF CLASS III ANTIARRHYTHMIC DRUGS

Gwilt, M.*, Higgins, A.J. and Williams, R.C. Pfizer Central Research, Sandwich Kent CT13 9NJ.

Two mechanisms of class III antiarrhythmic action have been studied extensively. These are blockade of the delayed outward $K^+$ current, $I_K$, e.g. by D-sotalol (Carmeliet, 1985) and delayed inactivation of the fast inward $Na^+$ current, e.g. by anaemonia sulcata toxin II ($ATX_{II}$) (Isenberg and Ravens, 1984). We have studied the effects of these drugs on refractoriness and repolarisation in rat and guinea pig ventricular muscle in vitro and in a model of acute myocardial ischaemia in anaesthetised rats.

Both D-sotalol (10-100 uM) and $ATX_{II}$ (1-5 nM) significantly P 0.05) prolonged the ventricular effective refractory period (ERP) in Langendorff perfused guinea pig hearts paced via the right ventricle. The magnitude of the $ATX_{II}$ effect depended critically on pacing rate. To produce a significant effect on ERP it was necessary to slow the spontaneous rate using carbamylcholine (10 uM) so that the pacing rate could be reduced from 4.5-5.0 Hz (control) to 2.5-3.0 Hz. Only $ATX_{II}$ (5 nM) prolonged ERP significantly in rat hearts. Similarly both D-sotalol and $ATX_{II}$ prolonged action potential duration (APD) in isolated guinea pig papillary muscles (1 Hz), while only $ATX_{II}$ prolonged APD in rat papillary muscles (1 Hz). These results are consistent with the absence or unimportance of $I_K$ in repolarisation in rat ventricle (Mitchell et al. 1984) and provide a simple method by which inhibitors of this current can be identified.

The effects of D-sotalol (10 mg/kg) and $ATX_{II}$ (5 ug/kg) on the incidence of arrhythmias following acute coronary ligation in the anaesthetised rat were also studied. $ATX_{II}$ prolonged the QT interval of the ECG and increased arterial pulse pressure (positive inotropy) for the duration of the experiment (30 min), as would be expected following delayed Na inactivation. No such effects were seen with D-sotalol. Neither drug reduced the number of extrasystoles, the incidence and duration of ventricular tachycardia, or the incidence of ventricular fibrillation and mortality following coronary artery ligation. The lack of antiarrhythmic effect with $ATX_{II}$ was surprising in view of its class III effects on rat ventricular muscle in vitro and clear evidence of this pharmacological action in vivo. A proarrhythmic action of $ATX_{II}$ (Isenberg and Ravens, 1984) may, however have offset its antiarrhythmic effects. Alternatively, the effects on repolarisation may not be uniform throughout the heart.

From these studies we conclude that:

1. Class III drugs which selectively reduce $I_K$ can be identified by their ability to increase APD and ERP in guinea pig but not in rat ventricle.

2. This makes the rat an unsuitable species for the routine testing of new antiarrhythmic drugs which may possess this mechanism.

3. Drugs which increase refractoriness in non-ischaemic myocardium do not necessarily prevent arrhythmias provoked by acute myocardial ischaemia in the rat.

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# THE EFFECTS OF THE THROMOBOXANE ANTAGONIST BM13.177 ON ISCHAEMIA AND REPERFUSION INDUCED ARRHYTHMIAS IN DOGS.

James R. Parratt and Cherry L. Wainwright*, Department of Physiology and Pharmacology, University of Strathclyde, 204 George Street, Glasgow, G1 1XW.

Previous studies with inhibitors of thromboxane synthesis (e.g. Coker, 1984) have led to the suggestion that thromboxane $A_2$ is involved in the genesis of ischaemia and reperfusion-induced arrhythmias. Further evidence for this suggestion has come from a recent study showing that the thromboxane antagonist AH23848 also protects against these arrhythmias Coker and Parratt, 1985). The aim of the present study was to determine the antiarrhythmic properties of the selective thromboxane antagonist BM13.177 (Müller-Beckman et al., 1983; Patscheke and Stegmeier, 1984) in a similar model of ischaemia and reperfusion in dogs.

Twenty greyhounds were anaesthetised with chloralose and prepared for occlusion of the left anterior descending coronary artery (LAD). Catheters were placed in the coronary sinus (draining the area rendered ischaemic by the occlusion) for determination of blood gases and pH. BM13.177 was given in a bolus dose of 5 mg $kg^{-1}$, 15 min prior to occlusion, and this was immediately followed by an infusion of 0.15 mg $kg^{-1}$ $min^{-1}$ which was maintained for the duration of the experiment. This dose of BM13.177 was found to abolish the haemodynamic responses to a bolus injection of 10 µg U46619.

BM13.177 markedly reduced the severity and incidence of arrhythmias resulting from both ischaemia and reperfusion. The number of ventricular actopic beats (VEB) during ischaemia was reduced from 1084 ± 159 in controls to 544 ± 179 ($P < 0.05$) in dogs given BM13.177. Similarly, 6 out of the 7 control dogs which survived the ischaemic period exhibited bursts of ventricular tachycardia (VT), whereas only 2 of the 9 surviving dogs given BM13.177 experience VT ($P < 0.01$). In total, 4 dogs fibrillated during ischaemia (3 control and 1 BM13.117). After 40 min of occlusion the ligature around the LAD was released. In the control dogs 6 of the remaining 7 fibrillated immediately upon reperfusion while the incidence of VF was 4 out of 9 in the BM13.177 ground ($P < 0.07$). The total incidence of VF during both ischaemia and reperfusion was reduced from 90% in controls to 50% in dogs given BM13.177 ($P< 0.05$).

These results lend further support to the theory that thromboxane is involved in the genesis of arrhythmias and that blockade of thrombozane receptors may be a suitable protective approach to antiarrhythmic therapy.

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# $\alpha_2$-ADRENOCEPTORS IN RAT FEMORAL VEIN

O. A. Downing, J. W. Smith, D. Stubbs* and K. A. Wilson. Pharmaceutical Sciences Institute, Aston University, Aston Triangle, Birmingham, B4 7ET.

The presence of post-junctional $\alpha_2$-adrenoceptors in the rat is well established in vivo. However, there are few reports of isolated blood vessels in which these receptors have been conclusively demonstrated (see Timmermans & van Zwieten, 1982). The present study describes preliminary attempts to characterise the $^{\alpha}$-adrenoceptors on the femoral vein of the rat.

5 mm long ring segments of femoral veins from male Wistar rats (180-220g) were mounted between two parallel fine steel wires in Krebs' solution at 37°C gassed with 95% $O_2$, 5% $CO_2$. The resting tension was 0.5 g. Three successive responses were elicited to each agonist ($10^{-6}$M) prior to construction of non-cumulative dose-response curves. Each agonist was left in contact with the tissue for 5 min and successive doses of agonist were applied at 20 min intervals. Noradrenaline (NA) $10^{-8}$M - $5 \times 10^{-6}$M, phenylephrine (PE) $10^{-8}$M - $5 \times 10^{-5}$M, UK-14,304 (UK) $5 \times 10^{-9}$M - $5 \times 10^{-7}$M and B-HT920 (BHT) $10^{-8}$M - $10^{-6}$M caused concentration dependent increases in tension. The $EC_{50}$ values for these agonists are shown in Table 1. The tissues were noticeably less sensitive to the $\alpha_1$-adrenoceptor agonist PE compared with the $\alpha_2$-adrenoceptor agonists UK or BHT or the non-selective $\alpha$-adrenoceptor agonist NA. Maximum responses to BHT, UK and PE were of similar magnitude and all were less than the maximum response to NA (see Table 1).

Table 1. Sensitivity of $\alpha$-adrenoceptor agonists on rat femoral vein

| | NA | BHT | UK | PE |
|---|---|---|---|---|
| $EC_{50}$ ($\times 10^{-8}$M) | 6.3 ± 0.7 | 14.3 ± 3.2 | 3.2 ± 0.4 | 45.5 ± 16.0 |
| $E_{max}$ (% NA) | 100 | 65 ± 8 | 63 ± 10 | 71 ± 12 |

Results are expressed as means ± s.e. mean

The time course of the contractile responses to each agonist was different. These differences were most marked at low doses. In particular the contractile responses to PE ($5 \times 10^{-8}$ to $5 \times 10^{-7}$M) were poorly maintained, the response falling to 50% or less of the peak values within the 5 minute period of drug contact.

The selective $\alpha_2$-adrenoceptor antagonist idazoxan (Doxey et al, 1983), $5 \times 10^{-8}$M - $10^{-6}$M, caused a parallel shift of the log dose-response curve to BHT to the right. The Schild plot for idazoxan against BHT was linear with a slope of 1.1 suggesting that the inhibition was competitive. The estimated $pA_2$ value was 7.2. In contrast, the $\alpha_1$-antagonist prazosin ($5 \times 10^{-9}$M) caused a slight but significant ($P<0.05$) shift of the log dose-response curve to BHT to the left.

These results suggest that the rat femoral vein possesses a significant population of postjunctional $\alpha_2$-adrenoceptors.

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# BENZODIAZEPINE AGONISTS AND INVERSE AGONISTS BOTH DECREASE LOCOMOTOR ACTIVITY AND BODY TEMPERATURE BUT SHOW MUTUAL ANTAGONISM.

Hilary J. Little, University Department of Pharmacology, South Parks Road, OXFORD, OX1 3QT.

Benzodiazepine agonists and inverse agonists have opposing effects in most tests, being anxiolytic and anxiogenic, anticonvulsant and convulsant, respectively. However, both groups of drugs decrease locomotor activity and lower body temperature in rodents (Taylor et al, 1985). These effects are antagonised by the benzodiazepine antagonist Ro 15-1788, but it is not clear whether the two groups of compounds exert their actions through similar mechanisms. The present data shows that, at certain doses, antagonism occurs between the two groups of drugs in these tests.

All experiments were carried out on male CD1 mice (Charles Rivers), 30-35g; injections were given i.p.. Locomotor activity was measured using pairs of mice; times given are from injection, except flurazepam which was given 15 minutes earlier. (The two sets of data were obtained using different types of meters). Body temperatures were measured by rectal probe, by an observer who did not know the drug treatment. All injections were made immediately after the first measurement of temperature, corresponding vehicles were given in addition to the single drug doses.

Locomotor Activity, mean ± s.e.m. (number of pairs).

| a) 0-30 min | | b) 0-60 min | |
|---|---|---|---|
| Vehicle (Tween 80) x 2 | 2343 ± 580 (5) | Vehicle (Tween 80) x 2 | 1939 ± 164 (6) |
| FG 7142, 40 mg $kg^{-1}$ | 1486 ± 327 (5) | FG 7142, 50 mg $kg^{-1}$ | 855 ± 80 (6) |
| Flurazepam, 5 mg $kg^{-1}$ | 384 ± 117 (5) | Diazepam, 2 mg $kg^{-1}$ | 406 ± 63 (6) |
| FG 7142 plus flurazepam | 2947 ± 1203 (5)* | FG 7142 plus diazepam | 1175 ± 208 (6)* |

| Body Temperatures, °C, mean ± s.e.m. (n) | Zero | 15 min | 30 min |
|---|---|---|---|
| a) Vehicle (8) | 37.3 ± 0.25 | 36.8 ± 0.33 | 36.9 ± 0.43 |
| FG 7142, 40 mg $kg^{-1}$ (8) | 37.4 ± 0.12 | 34.7 ± 0.32 | 35.3 ± 0.46 |
| Flurazepam, 5 mg $kg^{-1}$ (8) | 37.4 ± 0.19 | 35.4 ± 0.27 | 34.6 ± 0.28 |
| FG 7142 plus flurazepam (8) | 37.6 ± 0.22 | 36.6 ± 0.23*** | 36.6 ± 0.24*** |
| b) Vehicle (11) | 37.6 ± 0.22 | 37.4 ± 0.14 | 37.4 ± 0.13 |
| FG 7142, 50 mg $kg^{-1}$ (12) | 37.7 ± 0.1 | 34.5 ± 0.3 | 33.5 ± 0.36 |
| Diazepam, 2 mg $kg^{-1}$ (11) | 37.7 ± 0.14 | 34.6 ± 0.21 | 34.0 ± 0.29 |
| FG 7142 plus diazepam (12) | 37.6 ± 0.14 | 35.3 ± 0.14 | 35.0 ± 0.58** |

* $P<0.05$, compared with flurazepam alone. (Mann Whitney 'U' test).

** $P<0.05$, compared with FG 7142 alone.

*** $P<0.05$, compared with flurazepam or FG 7142 alone. (differences from controls not indicated).

A similar pattern was found with diazepam and CGS 8216 on body temperature. CGS 8216 is an inverse agonists which increases locomotor activity in mice (an effect antagonised by Ro 15-1788) but slightly lowers body temperature. When combined, other doses of FG 7142 and benzodiazepines showed neither additive not antagonist effects. The latter results resembled those found by File & Lister (1982) in exploratory head dipping and rearing in rats. The present results indicate that benzodiazepine agonists and inverse agonists act through different mechanisms to produce their effects on both locomotor activity and body temperature. In the case of the locomotor activity these mechanisms probably involve production of sedation by the agonists and "anxiety" by the inverse agonists. These might be expected to show mutual antagonism. Whilst locomotor activity affects body temperature, the two types of measurement did not change in parellel. It is possible that different receptors are involved in the hypothermic effects of benzodiazepine agonists and inverse agonists, but many mechanisms alter body temperature. GABA agonists and antagonists are both hypothermic, so the antagonism may occur at this level; pharmacokinetic interactions also need to be excluded before such speculation is justified.

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# THE EFFECTS OF DISCRETE INJECTIONS OF MUSCIMOL AND FLURAZEPAM INTO THE DORSAL RAPHE NUCLEUS ON TWO MODELS OF ANXIETY IN THE RAT

G.A. Higgins*, B.J. Jones and N.R. Oakley, Department of Neuropharmacology, Glaxo Group Research Ltd., Greenford, Middlesex, UB6 OHE.

The dorsal raphé nucleus (DR) is an important source of 5-HT containing neurones in the CNS. Recent evidence obtained by injecting drugs discretely into the DR suggests that it may have an important function in anxiety. For example, microinjection of chlordiazepoxide (CDP) in extremely low concentrations released punishment-suppressed responding in rats (Thiébot et al., 1982). Conversely, the benzodiazepine receptor inverse agonist, methyl-beta-carboline-3-carboxylate, also at low concentrations, was anxiogenic in the social interaction (SI) test (Hindley et al., 1985).

Both compounds are ligands for the benzodiazepine receptor, so they probably express their effects by modulating GABA function. Accordingly, we have examined the effects of the GABA agonist, muscimol, and the benzodiazepine, flurazepam, microinjected into the DR on two tests predictive of anxiolytic activity: the SI test (File, 1980) and the thirsty rat conflict test (TRC) (Vogel et al., 1971).

Male Lister Hooded rats (220-240g) were each implanted with a guide cannula under chloral hydrate anaesthesia so that the tip was 3mm above the DR. At least 7 days later, drugs were injected in 0.5µl artificial CSF into the DR using a fine glass capillary (Azami et al., 1980). Drug treatments were given 5 min before either test. The SI test was performed under high illumination over 10 min with both rats unfamiliar to the test arena. Only one rat of a pair was treated and assessed for SI. Locomotor activity (LMA) was recorded automatically. Rats were deprived of water for 18h for the TRC test. All injection sites were verified histologically.

Muscimol (5ng) significantly increased both SI (+81%, $P<0.05$) and LMA (+28%, $P<0.05$) relative to controls. Muscimol (10ng) also reversed the suppression of punished drinking in water deprived rats (mean shocks received: 27.5 ± 5.2; controls 6.8±2.2, $P<0.05$), without having any effect on free drinking. A lower dose of muscimol (5ng) produced a non-significant suppression (muscimol 30±7.6; controls 11.8±3.9. In contrast, flurazepam (0.05-10ng) had no significant effect on either the SI or TRC tests. A high dose (1µg) had an effect (+63%, $P=0.05-0.1$) in the SI test but not in the TRC test.

The clear effect of muscimol and the poor activity of flurazepam in this study suggest that in these rats at least, the normal GABA tone in the DR is insufficient for flurazepam to express its effects. Since Thiébot et al., (1982) observed a release of punishment-suppressed responding after intra-raphé CDP using Wistar rats, strain differences may account for this discrepancy. However, a more likely explanation is that the different experimental conditions affect the relative degrees of GABA tone (Gallager, 1978). This could explain why so many discrepancies occur in this type of work.

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# DIAZEPAM ANTAGONIST EFFECTS OF THE PYRAZOLOQUINOLINE CGS 9896

E. Morel, G. Perrault*, D.J. Sanger & B. Zivkovic (introduced by K.G. Lloyd). Laboratoires d'Etudes et de Recherches Synthelabo (L.E.R.S.), 31 Ave. P.V. Couturier, 92220 Bagneux, France.

Among a number of compounds recently shown to displace benzodiazepines from their binding sites, the pyrazoloquinoline, CGS 9896, has a particularly interesting pharmacological profile. This compound was originally reported to exert pharmacological effects indicative of clinical anxiolytic activity but not to produce depressant effects on behaviour even at very high doses, suggesting that it might be devoid of sedative and muscle relaxant activity in the clinic (Yokoyama et al 1982). More recently, it has been reported that, although CGS 9896 had little effect on the rotarod performance of rats, it was able to antagonise the deficit produced by diazepam in this test (Bernard et al 1985). The present series of experiments was therefore carried out in order to investigate the possibility that CGS 9896 might antagonise other pharmacological actions of diazepam.

Anticonvulsant effects were evaluated in male mice (CD1, 18-25 g) by observing the presence of tonic convulsions induced by pentetrazole (125 mg/kg, s.c.) or electroshock (60 mA, 50 Hz, 0.2 s) as well as by measuring the delay to the first convulsion after isoniazide (800 mg/kg, s.c.). The effects of drugs on locomotor activity and on the performance in the rotarod and loaded grid tests were measured in mice following the techniques described by Boissier and Simon (1966), Dunham and Miya (1957) and Fleury (1957), respectively. The staircase test and operant lever pressing were carried out with male rats (Sprague Dawley, 150-200 g) as described by Thiebot et al (1976) and Sanger et al (1985), respectively.

CGS 9896 completely prevented pentetrazole-induced seizures ($ED_{50}$ 4 mg/kg, i.p.) but was inactive against electroshock or isoniazide, whereas diazepam (0.1-10 mg/kg, i.p.) produced dose-related anticonvulsant effects in all procedures. CGS 9896 (30, 100 mg/kg, p.o.) gave rise to rightwards shifts in the diazepam dose-response curves against isoniazide and electroshock, but failed to affect its antipentetrazole activity. Also in mice, diazepam reduced locomotor activity, impaired rotarod performance and impaired the animals' ability to grip a metal grid loaded with weights. In these tests CGS 9896, at oral doses between 10 and 100 mg/kg, produced partial or complete antagonism of the effects of diazepam. In rats, diazepam (1-30 mg/kg) produced a dose-dependent decrease in exploratory locomotion (the staircase test) and in rates of operant lever pressing for food reward maintained by a fixed-ratio schedule. In both procedures the depressant effects of diazepam were antagonised by a dose of CGS 9896 (30 mg/kg, i.p.) which had little effect when administered alone.

These results demonstrate that CGS 9896 can antagonise anticonvulsant and central depressant effects of diazepam in both rats and mice. However, it is not yet clear whether CGS 9896 is best classified as a partial agonist at a single type of benzodiazepine receptor or as a mixed agonist-antagonist, acting as an agonist at certain receptor subtypes and as an antagonist at others.

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# DIFFERENCES BETWEEN THE STIMULUS PROPERTIES OF CHLORDIAZEPOXIDE AND ZOLPIDEM ARE REVEALED BY CGS 9896

D.J. Sanger* and B. Zivkovic (introduced by K.G. Lloyd). Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 31 Ave. P.V. Couturier, 92220 Bagneux, France.

Many recent studies have demonstrated that rats can learn to emit one response after administration of a benzodiazepine and another response after administration of the drug vehicle. Such stimulus control established with one benzodiazepine will generalise to other drugs in this class and is antagonised by Ro 15-1788 and CGS 8216, indicating an effect mediated by benzodiazepine binding sites. Non-benzodiazepines which act at the benzodiazepine binding site, such as zopiclone and CL 218,872, also produce benzodiazepine-appropriate responding.

Recently it was shown that zolpidem, a non-benzodiazepine which displaces benzodiazepine binding (Arbilla et al., 1985), produced relatively little drug lever responding in rats trained to discriminate between chlordiazepoxide (5.0 mg/kg) and saline using a standard, two-lever, operant conditioning procedure (Depoortere et al., 1986). However, in rats trained to discriminate zolpidem (2.0 mg/kg) from saline, chlordiazepoxide produced dose-related responding on the zolpidem lever although this generalisation occurred only at doses which decreased response rates (10, 20 mg/kg). In order to investigate the stimulus properties of chlordiazepoxide and zolpidem in more detail generalisation and antagonism tests were carried out with the pyrazoloquinoline CGS 9896.

Rats were trained to press one lever for food after a drug injection and a second lever after injection of saline. Two groups of animals were used, one trained to discriminate chlordiazepoxide at 5.0 mg/kg and the other trained to discriminate 2.0 mg/kg of zolpidem. As previoulsy described, (Sanger et al., 1985) rats trained with chlordiazepoxide showed generalisation to the drug lever after a variety of anxiolytic and potentially anxiolytic drugs including CGS 9896 (1-100 mg/kg). After combinations of chlordiazepoxide with a dose of CGS 9896 (30 mg/kg) all rats continued to respond on the chlordiazepoxide lever. Rats discriminating zolpidem generally responded on the saline lever after injection of CGS 9896 (10-100 mg/kg). However, when given with the training dose of zolpidem, CGS 9896 produced a dose-related (1-100 mg/kg) antagonism of the stimulus properties of this drug.

Thus, the stimulus properties of chlordiazepoxide generalised to CGS 9896 whereas those of zolpidem did not generalise and were antagonised by CGS 9896. It is known that CGS 9896 can exert both benzodiazepine-like agonist and antagonist effects in other test procedures. The present results suggest that the stimulus properties of chlordiazepoxide and zolpidem may be mediated by activity at different receptor subtypes. At one subtype CGS 9896 has agonist properties while at the other it acts as an antagonist.

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# CHARACTERISTICS OF [$^3$H]-(-)-NICOTINE BINDING TO RAT BRAIN

P.G. Jenner, R. Kumar, C.D. Marsden, C. Reavill* & I.P. Stolerman, Departments of Neurology, Pharmacology and Psychiatry, Institute of Psychiatry, London SE5 8AF.

Characteristics of [$^3$H]-(-)-nicotine binding to rat brain membranes have been studied, including its distribution across brain regions and the abilities of nicotinic and other drugs to inhibit the binding. The procedure was based on that for racemic nicotine (Marks & Collins, 1982). All samples were incubated in triplicate at 25°C for 10 min. For kinetic studies, specific binding of 0.5 - 64nM [$^3$H]- nicotine was defined by 10$\mu$M (-)-nicotine. For IC-50 studies, 4 nM [$^3$H]-(-)-nicotine was used to determine the relative potency of putative displacing agents in preparations of whole brain membranes.

In preparations of membranes from whole brains, high affinity [$^3$H]-(-)-nicotine and [$^3$H]-(±)-nicotine binding was saturable, and Scatchard analysis revealed evidence of one binding site ($r > 0.9$). For [$^3$H]-(±)-nicotine, $K_d$ = 12.0 ± 1.2 nM, $B_{max}$ = 90.4 ± 9.8 fmoles/mg protein; for [$^3$H]-(-)-nicotine, $K_d$ = 6.3 ± 1.5 nM, $B_{max}$ = 74.2 ± 8.1 fmoles/mg protein (means ± s.e.m.). Lower affinity binding was also detected but it was not saturable. Investigation of the regional distribution of [$^3$H]-(-)-nicotine binding sites revealed no differences in $K_d$. However, there were divergent values of $B_{max}$ in different areas with the thalamus having the largest concentration of binding sites (Table 1). Specific binding was 70 - 90% of total binding.

Table 1 Regional distribution of [$^3$H]-(-)-nicotine binding

$B_{max}$ values are fmoles/mg protein

| REGION | $K_d$ (nM) | $B_{max}$ |
|---|---|---|
| Thalamus | 7.8 ± 0.8 | 231 ± 19 |
| Cortex | 7.5 ± 1.7 | 143 ± 4 |
| Striatum | 7.2 ± 2.0 | 135 ± 12 |
| Pons-Medulla | 6.1 ± 0.8 | 75 ± 3 |
| Hippocampus | 6.6 ± 3.1 | 62 ± 4 |
| Hypothalamus | 8.2 ± 1.5 | 46 ± 2 |
| Cerebellum | 5.7 ± 0.3 | 39 ± 4 |

All the nicotinic-cholinergic agonists showed some activity as inhibitors of nicotine binding, whereas nicotine antagonists and other compounds were inactive. (-)-Nicotine was 13 times more potent than (+)-nicotine. Nornicotine, anabasine, cytisine and lobeline had IC-50 values in the nanomolar range, whereas morphine, phencyclidine, chlorisondamine, xanthine, uracil and procaine were generally inactive at 1 mM. Removal of the methyl group to form nornicotine abolished stereoselectivity.

The findings confirm the main characteristics of high-affinity nicotine binding which were determined with [$^3$H]-(±)-nicotine as the ligand in most previous studies (Marks & Collins, 1982; Romano & Goldstein, 1980). The data on regional distribution and on nicotine analogues may facilitate attempts to characterise the receptor mediating behavioural effects of nicotine.

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# BEHAVIOURAL EFFECTS OF THE OPTICAL ISOMERS OF NICOTINE AND NORNICOTINE, AND OF COTININE, IN RATS

H.S. Garcha, S.R. Goldberg, C. Reavill, M.E. Risner & I.P. Stolerman*, Departments of Pharmacology and Psychiatry, Institute of Psychiatry, London SE5 8AF, U.K. and National Institute on Drug Abuse, Addiction Research Center, P.O. Box 5180, Baltimore, Maryland 21224, U.S.A.

Tests of nicotine as a discriminative stimulus or on rates of rewarded responding can provide behavioural indices of its central actions. We report studies on some analogues and metabolites of nicotine. Groups of 8 rats were trained to discriminate natural (-)-nicotine (0.1 mg/kg) in a conventional, two-bar procedure with food reinforcement (Stolerman et al., 1984). A group of 5 rats was trained to press a single bar for food reinforcement delivered on a multiple fixed-interval 5 min, fixed-ratio 20 response schedule (Risner et al., 1985). All drugs were administered subcutaneously and doses are expressed as those of the bases.

In the nicotine discrimination procedure, (-)-nicotine increased the percentage of drug-appropriate responding in a dose-related manner and the $ED_{50}$ value was 0.045 mg/kg. The mean percentage of drug-appropriate responding was 84.5 $\pm$ 5.0% after nicotine (0.1 mg/kg) as compared with 5.6 $\pm$ 1.6% after saline (means $\pm$ s.e.m.). All nicotine analogues tested produced a full nicotine-like discriminative effect and the $ED_{50}$ values were 0.50 mg/kg for (+)-nicotine, 0.56 mg/kg for (-)-nornicotine, 0.37 mg/kg for (+)-nornicotine and 43 mg/kg for (-)-cotinine. Mean scores after maximally effective doses of the analogues were 79.6 - 87.5% (largest s.e.m. = 7.5%). Tests with morphine (1 - 4 mg/kg) and the beta-adrenoceptor agonist clenbuterol (0.1 - 3.2 mg/kg) confirmed that non-nicotinic compounds do not increase nicotine-appropriate responding in this procedure. In rats trained on the multiple schedule, the smaller doses tested of all compounds (except (+)-nicotine) increased overall rates of responding to 160 - 195% of control rates in the fixed-interval component only; larger doses of all compounds (except cotinine) decreased rates of responding in both schedule components. The rank order of potency of the compounds was the same as in the studies of nicotine discrimination.

The results extend to a wider range of procedures the evidence that the behavioural effects of nicotine are stereoselective (Meltzer et al., 1980). The low potencies of the metabolites (-)-nornicotine and (-)-cotinine support suggestions that they probably do not contribute to the effects of nicotine. The loss of stereoselectivity with nornicotine may have implications for the structure of CNS nicotinic receptors. The relative potencies of the compounds in the behavioural procedures were correlated with the concentrations that inhibited high-affinity binding of [$^3$H]-nicotine *in vitro* (Jenner et al., 1986), and thus the results support the view that this binding site functions as a receptor mediating behavioural effects of nicotine.

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# SEROTONERGIC MECHANISM FOR THE INHIBITORY EFFECT OF NON-BENZODIAZEPINE ANXIOLYTICS IN THE RAT HIPPOCAMPAL SLICE.

R. Anwyl[1] & M.J. Rowan*, Department of Pharmacology and Therapeutics, and [1]Department of Physiology, Trinity College, Dublin 2.

Buspirone and isapirone (TVX Q 7821) have anxiolytic properties but do not appear to interact with benzodiazepine receptors. They have been shown to possess high selectivity for 5-HT 1A binding sites in the hippocampus (Glaser & Traber, 1985; Peroutka, 1985). 5-HT is known to exert both excitatory and inhibitory effects on synaptically evoked firing of hippocampal pyramidal cells (Rowan & Anwyl, 1985). The present study compared the effects of the anxiolytics with the response to 5-HT in the rat isolated hippocampal slice preparation.

Transverse hippocampal slices (450μ thick) were dissected from male albino Wistar rats (150-200 g). The slices were superfused in a solution containing 120 mM NaCl, 2.5 mM KCl, 2.0 mM $CaCl_2$, 1 mM $MgSO_4$, 26 mM $NaHCO_3$, 1.25 mM $Na_2HPO_4$ and 10 mM glucose at 35°C and which was flowing at a rate of 10 ml/min. The stratum radiatum was stimulated at a frequency of 0.05 Hz and an extracellular population spike which was 50% of the maximum amplitude was recorded in the pyramidal cell layer of the CA1 region. Drugs were applied via the perfusion medium for 10 min.

In contrast to 5-HT, which typically produced an initial transient increase followed by a maintained decrease in the amplitude of the population spike, both anxiolytic compounds were purely inhibitory. Recovery to the control level after washout of these drugs took 4 to 5 times longer than following 5-HT application at similar concentrations. The inhibitory effect was found to be concentration-dependent in the range of 1-100μM. The three agents were of a similar order of potency. For example, 10 μM 5-HT produced a 57±11% inhibition (mean ± s.e. mean, n = 9) whereas 10 μM isapirone produced 37±8% inhibition (n = 6) and 10 μM buspirone produced 26±5% inhibition (n = 6). A strict comparison of potency was not possible because the inhibitory response to 5-HT was superimposed on the excitatory effect, which was also concentration-dependent in the same range. In four slices the population spike amplitude did not decrease below the control level in the presence of 10 μM 5-HT. These slices showed a reduced sensitivity ($P < 0.05$) to the inhibitory effect of both 10 μM isapirone (12 ± 2% inhibition) and buspirone (11 ± 4% inhibition).

The finding that both buspirone and isapirone mimicked the inhibitory effect of 5-HT on the population spike amplitude is consistent with 5-HT agonist action. The lack of excitatory effects may be considered to imply a degree of selectivity for one type of 5-HT receptor, that mediating inhibition. This hypothesis is supported by the observation that the compounds were much less active in slices which did not produce an observable inhibition in the presence of 5-HT. The present results support the proposal (Beck et al, 1985; Mason 1985) that the inhibitory effects of 5-HT in the hippocampus are mediated through 5-HT 1A receptors, since both buspirone and isapirone are potent and selective ligands for these sites.

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# PRAZOSIN ANTAGONISES THE WET-DOG SHAKE BEHAVIOUR PRODUCED BY INTRATHECAL ADMINISTRATION OF A TRH ANALOGUE, CG 3509

G.W. Bennett, K.C.F. Fone* & C.A. Marsden, Department of Physiology Pharmacology, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH.

The high density of thyrotrophin-releasing hormone (TRH)-like immunoreactivity in the ventral horn of the spinal cord (Kardon et al., 1977) and the ability of exogenous TRH to augment spinal reflexes (Clarke et al., 1984) suggests that TRH may be involved in spinal motor control. To investigate this proposed function, CG 3509 (orotyl-L-histidyl-L-prolineamide) was given by intrathecal (i.t.) injection to conscious rats and the motor component of the behavioural response examined.

Male Wistar rats were anaesthetized with sodium methohexitone (60 $mgkg^{-1}$ i.p.) and a polythene tube (0.5 mm O.D.) implanted along the spinal subarachnoid space (Yaksh & Rudy, 1976) such that the tip was at the thoraco-lumbar junction. After a seven day recovery period the number of wet-dog shakes (WDS) were monitored (between 08.00 and 12.00 h) during each minute for 30 min following 10 µl CG 3509 or saline i.t. washed in with 20 µl saline. Initially all rats received saline i.t. and at four day intervals thereafter either A. 0.5, 2, 10 and 20 µg CG 3509 in a randomized order followed by saline (n=6) or B. 2 µg CG 3509 30 min after vehicle i.p. and four days later 2 µg CG 3509 30 min after prazosin 2 $mgkg^{-1}$ i.p. (n=5). At the end of the dose response study (group A.), a cannula was implanted into the carotid artery under methohexitone anaesthesia and 24 h later the mean arterial blood pressure (b.p.) and heart rate monitored in the conscious animal following 10 µg CG 3509 i.t. These rats were given 5.9 KBq [$^{125}$I]TRH (10 µl i.t.) 24 h later then decapitated at the peak time of the CG 3509 response, i.e. 6 min after administration, and radioactivity counted in selected brain and spinal regions.

Compared with saline injection, CG 3509 produced an immediate dose-dependent increase in the number of WDS (mean ±s.e. mean in the first 6 min: saline, 0.2±0.2; 0.5 µg, 18.7±4.9; 2 µg, 32.3±9.7; 10 µg, 47.7±7.5 and 20 µg, 58.3±9.2 WDS) with a significant difference between each dose ($P<0.05$, analysis of variance). However, 10 and 20 µg CG 3509 produced a more transient effect than lower doses. Neither the b.p. nor the heart rate were significantly altered by 10 µg CG 3509 i.t., in contrast to the hypertension and tachycardia which occurs when TRH is given intracerebroventricularly (Tsay & Lin, 1982). After i.t. injection of [$^{125}$I]TRH there was ten times more radioactivity in the thoracic spinal cord than in the brain stem, further suggesting that the former may be the site of action.

The number of WDS in 30 min following 2 µg CG 3509 was significantly reduced by prazosin pretreatment (27.0±7.5 compared with 119.6±16.7, mean ±s.e. mean, $P<0.01$ Wilcoxon's rank sum test). This suggests that the WDS behaviour produced by i.t. CG 3509 may involve spinal catecholaminergic pathways which regulate motor activity.

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# 5-HT RELEASE AND METABOLISM IN RAT BRAIN DECREASED BY 8-OH-DPAT (IV): INVOLVEMENT OF 5-HT AND $\alpha_2$-ADRENORECEPTORS

C.A. Marsden & K.F. Martin*, Department of Physiology & Pharmacology, University of Nottingham Medical School, Clifton Boulevard, Nottingham NG7 2UH.

Radioligand studies have demonstrated the presence of multiple binding sites for 5-hydroxytryptamine (5HT) in brain (Peroutka & Snyder, 1979). The in vivo release and metabolism of 5HT in the suprachiasmatic nucleus (SCN) of the rat is decreased by the $5HT_{1B}$ agonist RU-24969 and this effect is mediated by autoreceptors located on nerve terminals (Marsden & Martin, 1985a). It has been suggested that the $5HT_{1A}$ agonist 8-OH-2-(n-dipropylamino)tetralin (DPAT) also has an agonist action at 5HT autoreceptors (Hjorth et al., 1982) although we found no evidence of this when DPAT was injected directly into nerve terminal or cell body regions (Marsden & Martin, 1985b). We now present in vivo evidence obtained using differential pulse voltammetry (DPV) that peripherally administered DPAT decreases 5HT release and metabolism in the SCN.

Male Wistar rats (290-310 g) were anaesthetised with chloral hydrate (600 mg/kg i.p.) and the left jugular vein cannulated for administration of drugs. A carbon fibre working electrode was stereotaxically-implanted into the left SCN. DPV's were obtained every 5 mins and the height of the oxidation peak at +300 mV ('peak 3') taken as a measure of the extracellular level of 5-hydroxyindoleacetic acid (5HIAA) (Sharp et al., 1984).

Administration of 0.9% saline (1 ml/kg i.v.) did not significantly alter the height of peak 3. However, after DPAT (0.1 mg/kg i.v.) peak 3 height decreased rapidly and significantly ($P<0.01$) by 22±3% (n=4) 30 min and 33±3% (n=4) 60 min post DPAT injection. When the non-selective 5HT receptor antagonist methiothepin (1 mg/kg i.v.) was given 5 min prior to DPAT this decrease was significantly ($P<0.05$) attenuated. Peak 3 height had decreased by 16±3% 60 min after DPAT preceded by methiothepin. The $5HT_2$ receptor antagonist ritanserin (2 mg/kg i.v.) also significantly ($P<0.05$) attenuated the effects of DPAT to an extent similar to that of methiothepin. Isapirone (1 mg/kg i.v.), a $5HT_{1A}$ antagonist, decreased the height of peak 3, but less than DPAT (17±6%, n=4, 30 min; 21±5, n=4, 60 min post injection). The response to DPAT was also significantly ($P<0.05$) less when isapirone was given 5 min prior to DPAT, 18+3% (n=4) 60 min post isapirone + DPAT c.f. 33±3%, 60 min post DPAT).

Idazoxan, an $\alpha_2$ receptor antagonist (0.1 and 0.2 mg/kg i.v.) did not significantly alter the height of peak 3 recorded in the SCN. However, after a dose of 0.5 mg/kg i.v. there was a large increase in peak 3 height which was greatest 45 min post-injection (119±12%, n=4). Idazoxan (0.2 mg/kg i.v.) given 5 min before DPAT completely abolished the decrease in peak 3 height observed when DPAT was given alone.

These data show that i.v. DPAT decreases 5HT release and metabolism in the SCN. This may involve $5HT_2$ and $\alpha_2$ receptors as well as the $5HT_{1A}$ receptor. Since we found that DPAT did not affect peak 3 height when injected into the SCN or dorsal raphe (Marsden & Martin, 1985b) the site of action remains unclear.

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# THE $\alpha_1$-ADRENOCEPTOR ANTAGONIST [$^3$H]WB 4101 LABELS 5-HT$_{1A}$ RECEPTORS, WITH NANOMOLAR AFFINITY.

D. Hoyer & H.O. Kalkman* (Introduced by G. Engel)
Preclinical Research, 386/525, SANDOZ Ltd., CH-4002 Basle, Switzerland

[3H]WB 4101 was introduced as a selective radioligand for $\alpha_1$-adrenoceptors (Greenberg et al., 1976). Several groups reported complex binding characteristics of this ligand and the existence of multiple affinity states of the receptor radioligand complex was suggested (Rehavi et al., 1980, Lyon and Randall, 1980, Weinreich and Seeman, 1981); Recently Creese and collaborators reported that [3H]WB 4101 labels $\alpha_1$-adrenoceptors with subnanomolar affinity and serotonin (5-HT$_1$) recognition sites with nanomolar affinity (Norman et al., 1985, Morrow et al., 1985). Since several 5-HT$_1$ receptor subtypes exist, we compared the binding profile of the various 5-HT recognition sites with those of [3H]WB 4101 binding performed in the presence of 30 nM prazosin (data from Norman et al., 1985 and Morrow et al., 1985). Radioligand binding studies were performed according to Hoyer et al (1985). 5-HT$_{1A}$: pig cortex, [3H]8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin). 5-HT$_{1B}$: [125I]CYP (iodocyanopindolol), in the presence of 30 µM isoprenaline in rat cortex. 5-HT$_{1C}$: [3H]mesulergine in pig choroid plexus. 5-HT$_2$: [3H]ketanserin in rat cortex. Table 1 lists the affinity values expressed as $pK_D$ or $pIC_{50}$ values (-log M), mean +/SEM.

| Table 1 | [3H]WB 4101 | 5-HT$_{1A}$ | 5-HT$_{1B}$ | 5-HT$_{1C}$ | 5-HT$_2$ |
|---|---|---|---|---|---|
| 5-HT | 8.2 | 8.5±0.1 | 7.6±0.1 | 7.5±0.1 | 7.1±0.3 |
| 8-OH-DPAT | 8.7 | 8.7±0.1 | 4.2±0.1 | 5.2±0.2 | 5.0±0.1 |
| Lisuride | 9.7 | 9.1±0.3 | 6.7±0.2 | 7.7±0.2 | 8.3±0.1 |
| 5-OCH$_3$-T | 7.0 | 8.0±0.2 | 6.4±0.2 | 7.4±0.3 | 5.6±0.3 |
| WB 4101 | 8.4 | 7.9±0.1 | 5.7±0.1 | 6.4±0.2 | 5.9±0.1 |
| Spiperone | 7.1 | 7.2±0.3 | 5.3±0.1 | 5.9±0.0 | 8.8±0.0 |
| Ketanserin | 5.9 | 5.9±0.2 | 5.7±0.1 | 7.0±0.1 | 8.9±0.1 |
| Phentolamine | 6.2 | 5.5±0.2 | 5.4±0.6 | 6.1±0.4 | 6.0±0.0 |
| Prazosin | 5.9 | 5.0±0.2 | 5.1±0.3 | 4.7±0.2 | 4.0±0.2 |
| d-LSD | 8.3 | 8.6±0.1 | 6.8±0.3 | 7.9±0.1 | 8.6±0.1 |

WB 4101 shows high affinity for $\alpha_1$ and 5-HT$_{1A}$ binding but low affinity for other 5-HT recognition sites. The pharmacological profile of the sites labelled with [3H]WB 4101 and [3H]8-OH-DPAT is very similar. There is a highly significant correlation between their affinity values: slope = 1.04, r = .923, P = .001. In contrast [3H]WB 4101 binding is very different from 5-HT$_{1B}$, 5-HT$_{1C}$ and 5-HT$_2$ binding as illustrated by the lack of correlation with these sites: r and P values ranged from .192 to .402 and from .59 to .25 respectively.
This data clearly demonstrates that the serotonergic component of [3H]WB 4101 binding is to 5-HT$_{1A}$ recognition sites as suggested by Morrow et al, 1985. The fact that [3H]WB 4101 is able to bind with high affinity to both, $\alpha_1$ and 5-HT$_{1A}$ receptors provides a simple explanation for the complex binding behaviour reported for this radioligand in various membrane preparations.

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# BEHAVIOURAL RESPONSES TO THE D-1 DOPAMINE RECEPTOR AGONIST SK&F 38393 AND THE D-2 AGONIST RU 24213 ALONE AND IN COMBINATION.

Marcellina Mashurano & J.L.Waddington*, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, St.Stephen's Green, Dublin 2.

The ability of the selective D-1 dopamine receptor antagonists SCH 23390 and SK&F 83566 to block potently a variety of responses previously equated with D-2 receptor function (Molloy & Waddington, 1984, 1985b; Pugh et al, 1985) has prompted renewed interest in the functional role of the D-1 receptor. The D-1 agonist SK&F 38393 fails to induce typical stereotyped behaviour, though it can induce episodes of a variety of subtler, non-stereotyped responses, especially prominent grooming (Molloy & Waddington, 1984, 1985a). We have also found it difficult to induce compulsive, fixated stereotyped behaviour even with high doses of the selective D-2 agonist RU 24213 (Pugh et al, 1985), though these are typical responses to the non-selective agonist apomorphine. To investigate whether these distinctions might reflect the absence and presence of D-1 agonist activity, we have studied behavioural responses to SK&F 38393 and RU 24213, given alone and in combination.

Male Sprague-Dawley rats were given two s.c. injections 10 min apart: vehicle + vehicle; SK&F 38393 + vehicle; vehicle + RU 24213; SK&F 38393 + RU 24213 (each, $n = 8$). At 10 min intervals thereafter they were each assessed for 1 min using a stereotypy rating scale combined with a behavioural check list, for a period of 1 h (Pugh et al, 1985).

15 mg/kg SK&F 38393 failed to induce stereotyped behaviour (mean score at 40 min: $1.4 \pm 0.2$), while 2.5 mg/kg RU 24213 was a threshold dose for inducing bursts of stereotyped sniffing and locomotion ($1.9 \pm 0.2$). Responses to this dose of RU 24213 were dose-dependently increased in combination with 3 mg/kg ($2.3 \pm 0.3$)-15 mg/kg SK&F 38393 ($3.1 \pm 0.4$; $p<0.05$ vs. either drug given alone). In the single treatment groups, only 1 rat given RU 24213 showed continuous sniffing with or without locomotion, while 6 showed such a response when combined with 15 mg/kg SK&F 38393 ($p< 0.05$); over the period of peak drug effect (30-60 min), compulsive stereotyped behaviour (i.e. fixated sniffing with or without licking/gnawing) was not seen in either single treatment group while 4 animals ($p<0.05$) given this combination showed such a syndrome.

The present results complement those showing that responses to the selective D-2 agonist RU 24213 can be blocked by the selective D-1 antagonists SCH 23390 and (enantioselectively) R- but not S- SK&F 83566 (Molloy & Waddington, 1985b). Decreases and increases in tonic D-1 activity appear to exert inhibitory and facilitatory influences on the intensity of behaviours initiated through D-2 stimulation. This supports the notion (Pugh et al, 1985) of a general 'enabling' function for D-1 tone in the expression of D-2-mediated responses in the whole animal.

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# HYPER-RESPONSIVENESS TO L-DOPA FOLLOWING MPTP TREATEMENT IN THE MARMOSET: MODIFICATION BY DEPRENYL

N.J.G. Barnes*, Brenda Costall & R.J. Naylor, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP.

1-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) given systemically or directly into the nigrostriatal dopamine system in the primate induces a syndrome resembling that of Parkinson's disease in man (Barnes et al, 1985). In the present study we investigate the possibility that the actions of MPTP may be associated with changed responsiveness to l-dopa, and that such changes may be prevented by deprenyl.

Common marmosets (Callithrix jacchus, male, 350-400g) were given (i.p.) (a) MPTP, 4 mg/kg on day 1, 2 mg/kg on days 2, 3 and 4, or vehicle, (b) MPTP + deprenyl (1 mg/kg daily throughout the MPTP treatment and on the day prior to treatment). n = 4 for each group. Spontaneous locomotor activity (measured in cages having 4 computer linked infrared units strategically placed to allow the detection of movement about the cage) was assessed for a 1h period during MPTP treatment, and then at weekly intervals for 11 weeks. 4 days after discontinuing treatments with MPTP or MPTP/deprenyl marmosets were challenged with l-dopa (6.25-100 mg/kg i.p. given 30 min after benserazide, 12.5 mg/kg i.p.) and locomotor activity was measured for a 2h period. Throughout all test periods marmosets were also visually observed via remote video recording.

Control recordings of spontaneous locomotion, taken on 4 occasions prior to drug treatment, were consistent (10-20 counts/10 min). Recordings taken 7h after recovery from the initial effects of MPTP (to cause prostration) showed MPTP alone to cause marked hypokinesia (locomotor activity reduced by approximately 90%, $P<0.001$, which was maintained throughout the 4 days of treatment). During the subsequent 7-22 days the hypokinesia persisted (80-95% reductions in locomotion, $P<0.001$), tremor was observed in the front and/or hind limbs, and muscular rigidity was evident on handling. However, between 3-5 weeks following the MPTP treatment spontaneous locomotion gradually returned towards control values, although tremor and rigidity were still clearly evident. The administration of deprenyl to marmosets before and during the MPTP treatment lead to significant reductions in the motor disturbances described for MPTP treatments alone. Within 3 days of ceasing MPTP/deprenyl treatment locomotor activity returned to control levels which were maintained for the duration of the experiment. Also, in the deprenyl treated animals, MPTP failed to cause tremor or rigidity.

L-dopa (12.5-100 mg/kg, in the presence of benserazide) failed to modify the locomotor responding of 'normal' marmosets. However, those marmosets which had received MPTP treatment responded to l-dopa (12.5 mg/kg) with marked hyperactivity (40-60 counts/10 min), developing within 30-40 min of treatment and persisting for 2h. Higher doses of l-dopa caused excessive 'excitement', and these experiments were terminated. In contrast, those marmosets which had received deprenyl in combination with MPTP failed to show any hyperactivity responding to l-dopa (data indistinguishable from that of control animals, $P>0.05$).

It is concluded that in the marmoset MPTP can cause a severe hypokinesia, associated with the development of enhanced responsiveness to l-dopa, and that such actions can be prevented by co-treatment with deprenyl.

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# FUNCTIONAL ACTIVATION OF DOPAMINE $D_1$ AND $D_2$ RECEPTORS BY ENDOGENOUSLY-RELEASED DOPAMINE IN RAT STRIATUM

E. Kelly* & S.R. Nahorski, Department of Pharmacology & Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester. LE1 7RH.

In rat striatum, dopamine D-1 and D-2 receptors are linked in opposite ways to the enzyme adenylate cyclase (Stoof & Kebabian, 1981). D-1 receptors mediate an increase in intracellular cyclic AMP accumulation whereas D-2 receptors mediate inhibition of D-1 stimulated cyclic AMP accumulation (Lazareno et al. 1985). Previous experiments have utilised exogenously administered drugs, but here we show that endogenous dopamine released by amphetamine can activate both striatal D-1 and D-2 receptors in vitro.

The cyclic AMP content of striatal slices (0.3 x 0.3 mm) was estimated in the presence of 1 mM 3-iso-butyl-1-methyl xanthine using a protein binding assay. The dopamine content of the incubation medium was measured by HPLC with electrochemical detection. In striatal slices, amphetamine (1-100 µM) caused a small increase in cyclic AMP accumulation (Table 1). Inclusion of the selective D-2 antagonist (-)-sulpiride (30 µM) in the incubation medium greatly enhanced the response to amphetamine (Table 1). The increase in cyclic AMP due to amphetamine plus (-)-sulpiride was not due to an enhancement of stimulated dopamine release, since measurement of the dopamine concentration in the incubation medium indicated that amphetamine (1-100 µM) produced up to a 4-fold increase in dopamine, but that this was not further increased by 30 µM (-)-sulpiride. The ability of amphetamine plus (-)-sulpiride to enhance cyclic AMP accumulation in slices was abolished by the selective D-1 receptor antagonist SCH 23390 (1 µM). Furthermore, amphetamine's action was due to release of endogenous dopamine rather than a direct action of the drug at D-1 receptors since i) amphetamine (1-500 µM) did not stimulate cyclase activity in striatal membranes, and ii) pre-treatment of animals with reserpine (5 mg/kg, s.c., 18 h previously) and slices with α-methyl-p-tyrosine (50 µM, 90 min) greatly reduced amphetamine-induced dopamine release and cyclic AMP accumulation.

Table 1

| | 0 | $10^{-6}$ M | 3 x $10^{-6}$ M | $10^{-5}$ M | $10^{-4}$ M |
|---|---|---|---|---|---|
| Amphetamine alone | 100 | 105 ± 6 | 103 ± 4 | 124 ± 8 | 140 ± 7 |
| + 30 µM (-)-Sulpiride | 101 ± 3 | 110 ± 9 | 139 ± 9* | 180 ± 9* | 195 ± 19* |

% Increase in cyclic AMP accumulation in striatal slices due to amphetamine in the presence or absence of (-)-sulpiride. Basal levels of cyclic AMP ranged from 8.07 to 10.64 pM/mg protein. n = 4 separate experiments, * $p < 0.05$ compared to amphetamine alone, paired 't' test.

These results clearly indicate for the first time, that endogenous dopamine released by amphetamine can functionally activate both D-1 and D-2 receptors in striatum in vitro.

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# ACUTE AND REPEATED ADMINISTRATION OF SULPIRIDE ALTERS MET- AND LEU-ENKEPHALIN CONTENT OF RAT BRAIN

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

Repeated, but not acute, haloperidol treatment increased met-enkephalin content in the striatum and nucleus accumbens of the rat (Hong et al 1978). However, the atypical neuroleptic clozapine failed to alter the striatal enkephalin content. We now report the effects of acute and repeated administration of the atypical neuroleptic sulpiride, on met- and leu-enkephalin levels in a variety of dopaminergic areas of brain.

Male Wistar rats (200-220g at the start of the experiment) were used in all studies. In acute experiments animals received sulpiride (100 mg/kg ip) 3h prior to death. In repeated administration experiments animals received sulpiride (10 or 100 mg/kg ip) for 21 days and were killed 2 days later. Peptides were extracted from dissected brain areas, and measured by means of selective radioimmunoassays after HPLC separation.

Acute administration of sulpiride (100 mg/kg ip) increased met-enkephalin levels in the striatum but decreased those in the substantia nigra and nucleus accumbens. No effect was observed in the hypothalamus or cortex. In contrast, leu-enkephalin content was decreased in the nucleus accumbens but not elsewhere. Administration of sulpiride (10 or 100 mg/kg ip) for 21 days followed by 2 days drug withdrawal resulted in an increase in met-enkephalin content of the nucleus accumbens but decreases in both the hypothalamus and frontal cortex. No change was observed in the met-enkephalin content of the striatum or substantia nigra. Repeated sulpiride administration caused a decrease in leu-enkephalin content of the hypothalamus and cortex but not elsewhere.

Table 1 Enkephalin levels following sulpiride (100 mg/kg ip) administration

| | Striatum | N.Accumbens | Hypothalamus | S.Nigra | Cortex |
|---|---|---|---|---|---|
| | | | met-enkephalin (pmol/g tissue) | | |
| Acute | | | | | |
| Control | 574.5±38.0 | 342.2±27.7 | 445.7±25.4 | 94.3±6.8 | 47.2±5.6 |
| Sulpiride | 829.6±80.8* | 113.8±13.1* | 367.3±24.7 | 62.9±10.0* | 59.2±7.6 |
| Repeated | | | | | |
| Control | 502.0±21.0 | 297.2±31.2 | 320.2±25.6 | 88.7±5.6 | 48.2±5.2 |
| Sulpiride | 491.8±43.8 | 430.0±26.2* | 168.0±21.0* | 83.8±10.5 | 28.7±1.7* |
| | | | leu-enkephalin (pmol/g tissue) | | |
| Acute | | | | | |
| Control | 63.4±7.9 | 112.7±17.6 | 56.4±5.2 | 108.4±10.2 | 62.7±7.6 |
| Sulpiride | 72.8±7.1 | 35.7±4.3* | 63.4±7.5 | 113.3±10.4 | 51.7±7.0 |
| Repeated | | | | | |
| Control | 75.4±4.5 | 108.6±10.6 | 69.2±5.5 | 85.6±6.4 | 53.7±4.8 |
| Sulpiride | 60.9±6.6 | 120.6±5.2 | 33.4±3.5* | 66.0±7.8 | 29.9±1.9* |

* $p < 0.05$ compared to controls; n = 5-6

Acute administration of sulpiride, unlike haloperidol, alters brain enkephalin levels. Similarly, in contrast to haloperidol, repeated sulpiride treatment does not alter the enkephalin content of the striatum or substantia nigra. However, both drugs increase the enkephalin content of the nucleus accumbens and this may relate to their common antipsychotic activity.

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# STUDIES OF THE EFFECTS OF IDAZOXAN ON CORTICAL NORADRENALINE RELEASE IN VIVO, MEASURED BY TRANSCORTICAL DIALYSIS

C.J. Carter, T. Dennis*, R. L'Heureux and B. Scatton. Biochemical Pharmacology Group, Laboratoires d'Etudes et de Recherches Synthélabo, 31 Avenue Paul Vaillant Couturier, 92220 Bagneux, France.

In the central nervous system (CNS), the presence and functional importance of presynaptic $\alpha_2$-adrenoceptors has been difficult to demonstrate as destruction of cerebral noradrenergic neurones fails to reduce $^3$H-clonidine or $^3$H-idazoxan binding in cortical membranes (U'Prichard et al., 1979 ; Pimoule et al., 1983). Idazoxan does however increase the turnover and release of cortical NA in vivo when administered systemically (Scatton et al., 1983 ; L'Heureux et al., 1986). The relative importance of presynaptic, postsynaptic or somatodendritic $\alpha_2$-receptors in producing this effect is unclear and we have now investigated this problem using a transcortical dialysis technique.

In anaesthetised rats, implanted with Diaflo fibre (Amicon) collecting from the lateral extent of the temporal cortex (A:0 ; V:-2mm ; Paxinos and Watson, 1982) idazoxan (20 mg/kg, i.p.) increased cortical NA efflux by 5 fold 40 min after injection (basal efflux values = 8.7 $\pm$ 0.3 pg/20 min fraction at a perfusion rate of 2 µl/min). The effects of idazoxan were potentiated by desipramine (DMI) pretreatment (20 mg/kg, i.p., 40 min before idazoxan). DMI alone produced a 2 fold increase in cortical NA output which was sustained for 4 hr. In DMI-pretreated rats, idazoxan (20 mg/kg, i.p.) produced a 24 fold increase in NA efflux 40 min after injection. The potentiation by DMI of the idazoxan response is likely due to the higher synaptic levels of NA producing tonic $\alpha_2$-receptor stimulation, against which the effects of $\alpha_2$-receptor antagonism may be better expressed. Cortical NA efflux was also increased four fold when idazoxan ($10^{-4}$M) was infused into the cortex via the dialysis fibre itself.

In awake rats, implanted with a transcortical dialysis fibre, basal NA efflux was stable for a period of four days. When administered on the first day after implantation of the dialysis fibre, idazoxan (20 mg/kg, i.p.) +DMI (20 mg/kg, i.p.) produced a 40 fold increase in cortical NA efflux 40 min after injection. The same rats then received an infusion of ibotenic acid via the cortically implanted dialysis fibre (10 µg/µl at 2 µl/min for 20 min). This lesion produced a zone of near total cortical neuronal destruction within a 2.5 mm radius from the dialysis fibre. On day 4, systemic injection of idazoxan (20 mg/kg, i.p.) +DMI (20 mg/kg, i.p.) to ibotenate-lesioned rats produced a 38 fold increase in cortical NA efflux 40 min after injection.

In conclusion, the persistence of the effect of idazoxan on cortical NA release after ibotenic acid induced lesions of NA target cells suggests that postsynaptic $\alpha_2$-adrenoceptors play a relatively minor role in regulating central NA release. This data, and the fact that intracortical infusion of idazoxan does increase cortical NA release suggest that presynaptic $\alpha_2$-receptors are important in mediating the action of idazoxan, despite their low density.

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# DIFFERENTIAL DISTRIBUTION OF ADRENORECEPTORS IN THE HEPATIC ARTERY AND PORTAL VEIN OF THE RAT

P N Bennett, Lidia J Notarianni and A J Worlock*
School of Pharmacy and Pharmacology, University of Bath, BA2 7AY

It has been reported that there is a lack of β-adrenoreceptors in the portal vein (PV) of the dog whereas the hepatic artery (HA) contains both α- and β-receptors (Richardson, 1982). The HA flow may therefore be pharmacologically altered without a similar change in PV flow, modifying the PV:HA ratio. We have examined the adrenoreceptors in the HA and PV of the rat since we have shown that lignocaine metabolism is dependant on this ratio (Bennett et al, 1984).

Male Wistar albino rats weighing between 400 and 450 g were anaesthetised with pentobarbitone (70 mg/kg i.p.). The PV and HA were cannulated and the livers perfused with Krebs Medium containing human red blood cells at a haemoglobin concentration of 5-6% (wt/vol). Perfusate from the liver drained from a cannula in the vena cava. Liver blood flow was constant at 2.5 ml/min through the HA and 7.5 ml/min through the PV which approximates to physiological flow.

Introduction of Noradrenaline (NA) either to the HA or PV resulted in a dose-related increase in pressure. A plot of change in pressure against $\log_{10}$NA concentration was sigmoidal. Phentolamine was added to the perfusion medium to antagonise the NA-induced vasoconstriction and the concentration now required to give 50% of the maximum pressure (ED-50) established.

Isoprenaline (ISO) introduced into the HA or PV produced no change in perfusion pressure. However, if ISO was added prior to NA it physiologically antagonised the NA vasoconstriction. The concentration of NA now required to give the ED-50 was used as an indicator of β-receptor activity. To further categorise the β-receptors, the liver was infused with $\beta_1$- and $\beta_2$- specific blockers before ISO and NA. Some results are given below.

Table 1: Concentration of NA (x10-$^6$M) required to give 50% of the maximum pressure in the HA and PV in the presence of various adrenoceptor agents.

| | HEPATIC ARTERY | | | PORTAL VEIN | | |
|---|---|---|---|---|---|---|
| | []x10$^{-5}$M | NA(ED-50) | n | []x10$^{-5}$M | NA(ED-50) | n |
| Control | | 0.293±0.01 | 10 | | 1.33± 0.23 | 10 |
| Phentolamine | 0.5 | 5.71 ±0.15 | 4 | 1 | 24.5 ±10.0 | 5 |
| ISO | 5.0 | 1.29 ±0.09 | 6 | 1 | 10.2 ± 0.90 | 5 |
| ISO + Atenolol | 5.0+0.5 | 0.271±0.04 | 3 | 1+1 | 5.89± 1.05 | 3 |
| ISO+ICI 118551 | 5.0+0.5 | 1.02 ±0.18 | 3 | 1+1 | 1.58± 0.21 | 3 |

Differences exist in the adrenoceptor population in the HA and PV of the rat. Both vessels contain α- and β- adrenoceptors, the HA being more sensitive to α-agonism/antagonism than the PV. Reversal of the ISO effect was achieved with both $\beta_1$ and $\beta_2$ antagonists. In the PV the $\beta_2$ selective antagonist (ICI 118551) was more effective than the $\beta_1$ antagonist (atenolol). In the HA the reverse was true, ICI 118551 being less effective than atenolol. The results indicate that $\beta_2$ receptors predominate in the vein whereas the $\beta_1$ receptor population may be greater in the artery.

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# EFFECTS OF ADENOSINE, ATP AND NORADRENALINE ON NORMAL AND MILDLY 'ISCHAEMIC' SHEEP CARDIAC PURKINJE ACTION POTENTIALS

G. Boachie-Ansah, K.A. Kane & J.R. Parratt, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW.

The effects of adenosine, adenosine triphosphate (ATP) and noradrenaline on the intracellular action potential characteristics of sheep Purkinje cells superfused in vitro with a normal or an altered physiological salt solution (P.S.S.) were examined. The P.S.S. (normal composition in mM: NaCl 125, KCl 5.4, $NaHCO_3$ 25, $NaH_2PO_4$ 1.2, $MgCl_2$ 1.8, glucose 5.5) was altered to mimic some of the conditions occurring during myocardial ischaemia by raising the potassium concentration to 8 mM, lowering the pH from 7.4 to 6.8 U and the $PO_2$ in the organ bath from 400 to <40 mmHg. The preparations were stimulated at a frequency of 1.5 Hz. In the experiments using normal P.S.S., the drug was added cumulatively and readings taken from 10 cells before and after a 30 min exposure period. A different set of preparations was exposed to either altered P.S.S. alone and readings taken prior to and after 30, 60, and 90 min exposure or to altered P.S.S. alone for 30 min followed by that containing the drug at 2 different concentrations; each drug exposure period being 30 min.

Exposure to modified P.S.S. alone significantly reduced the resting membrane potential, RMP, (86.3±0.6 to 76.8±1.6 mV), the action potential amplitude, APA, (119.1±1.6 to 87.2±4.2 mV), the maximum rate of depolarisation of phase zero, MRD, and the action potential duration at 90% repolarisation, $APD_{90}$ (see Figure 1).

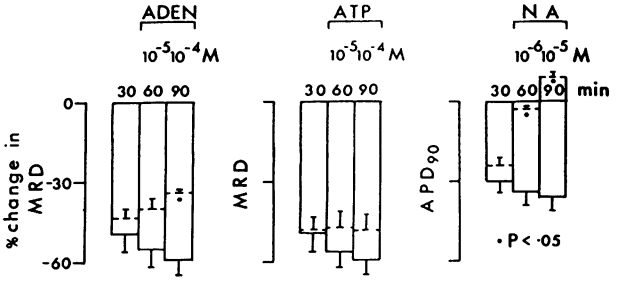


Figure 1 compares the % decrease in MRD and in $APD_{90}$ under conditions of altered P.S.S. (unbroken line) alone and altered P.S.S. in combination with adenosine and ATP (MRD only) and with noradrenaline ($APD_{90}$) only (broken line)

Adenosine significantly attenuated the fall in MRD induced by altered P.S.S. and the effect of ATP although similar was less marked and not statistically significant. The reduction in APA but not that in RMP or APD was also attenuated by both drugs. Neither adenosine nor ATP in these concentrations significantly enhanced MRD or normal Purkinje cells. Noradrenaline completely reversed the shortening in action potential duration induced by conditions that mimic ischaemia without influencing the other measured variables. In normal P.S.S., noradrenaline lengthened the action potential duration but to a lesser extent than in altered P.S.S.

These results show that the electrophysiological effect of adenosine and noradrenaline under ischaemic conditions differ from those observed on normal Purkinje cells.

# EFFECT OF CENTRAL AND SYSTEMIC ADMINISTRATION OF A VASOPRESSIN $V_1$ ANTAGONIST ON RECOVERY OF MAP AFTER HYPOVOLAEMIA IN RATS

G.W. Bennett, R. Hatton[1] & J.V. Johnson*, Department of Physiology & Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH and [1]ICI Pharmaceuticals Division, Alderley Park, Macclesfield SK10 4TG.

Haemorrhage is a potent stimulus for vasopressin (AVP) release and it has been shown that AVP may play a role in the recovery of blood pressure following acute haemorrhage in anaesthetised rats (Zerbe et al., 1982). The present investigation studied the effect of the AVP $V_1$ antagonist, $d(CH_2)_5Tyr(Me)AVP$, on the recovery of mean arterial blood pressure (MAP) in conscious rats following controlled haemorrhage (25% of calculated blood volume over 5 min via a carotid arterial catheter). The AVP antagonist was administered either intracerebroventricularly (i.c.v.) or intravenously (i.v.) at 5 min pre- or 15 min post-haemorrhage and the MAP monitored for 45 min post-haemorrhage. The rats were then killed and the neurointermediate lobe (NIL) and selected hypothalamic regions dissected out for AVP measurement by the technique of HPLC with electrochemical detection (Johnson et al., 1984).

The initial fall in MAP (from 132.1±2.2 to 72.2±4.4 mmHg) following haemorrhage was identical in the eight groups studied (n=8 in each group). Following administration of $d(CH_2)_5Tyr(Me)AVP$ (at 10 $\mu gkg^{-1}$ and 100 $ngkg^{-1}$) i.v., there was no significant effect on MAP recovery compared with saline treated controls up to 20 min post-haemorrhage. Thereafter the rats administered AVP antagonist at 10 $\mu gkg^{-1}$ had significantly lower MAP than control animals ($P<0.05$ at 25 min; $P<0.01$ at 45 min) but this attenuated effect was not seen with $d(CH_2)_5Tyr(Me)AVP$ at 100 $ngkg^{-1}$. In contrast with the lack of effect of the lower dose of the AVP antagonist given i.v., administration of 100 $ngkg^{-1}$ $d(CH_2)_5Tyr(Me)AVP$ i.c.v. caused an attenuated blood pressure recovery to haemorrhage comparable with that obtained with the antagonist at 10 $\mu gkg^{-1}$ i.v. In addition, rats administered the AVP antagonist (10 $\mu gkg^{-1}$) i.v. followed by naloxone (10 $ngkg^{-1}$) i.v. at 15 and 20 min post-haemorrhage respectively, showed a significantly improved MAP recovery by 45 min post-haemorrhage compared with the rats administered the AVP antagonist alone ($P<0.05$) although MAP was still lower than saline treated rats ($P<0.05$).

Haemorrhage caused a fall in AVP levels in the NIL and median eminence and a rise of AVP levels in the hypothalamus at 45 min post-haemorrhage compared with non-haemorrhage controls but these changes did not reach significance and were unaffected by administration of the AVP antagonist.

The results indicate a role for AVP in blood pressure recovery following haemorrhage which may be centrally mediated, although no apparent function in limiting the initial fall of blood pressure. In the presence of AVP blockade, naloxone improved the MAP recovery to haemorrhage suggesting that endogenous opioids may act to oppose the recovery of MAP to haemorrhage. The small changes in tissue AVP levels suggest some increase in AVP release following haemorrhage and this is consistent with the findings of Feuerstein et al. (1984) who showed highest plasma AVP levels immediately post-haemorrhage but no significant decrease of AVP in the NIL until 24 hs post-haemorrhage.

JVJ is an SERC CASE Award student with ICI.

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# FISH NEUROPEPTIDE UROTENSIN II CONTRACTS RAT AORTA SEGMENTS IN VITRO

H. Itoh, K. Lederis* & J. Rivier[1], Department of Pharmacology & Therapeutics, University of Calgary, Calgary, Alberta T2N 4N1 and [1]The Salk Institute, San Diego, CA 92037

Recent reports confirm a previous observation that urotensin II (UII), a peptide isolated from the caudal neurosecretory system of several species of bony fishes (Pearson et al, 1980; McMaster & Lederis, 1983; Ichikawa et al, 1984), induces vascular and other smooth muscle effects in mammals (Muramatsu et al, 1979; Gibson et al, 1984; Bern et al, 1985; Itoh et al, 1985).

In the present investigation, contractile effects of UII were determined and analyzed in helical strips of different regions of the aorta and in other major arteries of the rat. The upper segment of the descending thoracic aorta and the carotid artery were the most sensitive, the mesenteric artery a moderately sensitive preparation and the femoral artery not responsive at all to UII at $10^{-10}$M, $10^{-9}$M and $10^{-8}$M, respectively.

Agents which modify cyclic nucleotide levels in smooth muscle cells by different mechanisms, i.e., papaverine, dibutyryl cyclic AMP, forskoline and nitroprusside, all decreased the UII responses of the descending thoracic aorta strips ($IC_{50}$= $7.6x10^{-6}$M, $2.1x10^{-4}$M, $2.5x10^{-6}$M and $1.5x10^{-8}$M, respectively). Verapamil antagonized only partially the UII response at $IC_{50}$=$6.5x10^{-6}$M; complete inhibition (relaxation induced by 0.1M papaverine=100% inhibition) could not be obtained at $3.5x10^{-5}$M verapamil. In contrast to the above, phentolamine ($10^{-5}$M), propranolol ($10^{-5}$M), atropine ($10^{-4}$M), tetrodotoxin ($10^{-6}$ M), burimamide ($10^{-5}$M) and indomethacine ($10^{-5}$M) did not change significantly the UII induced contractile responses. The response was also not changed by removal of endothelial cells from the aortic strips. Only minimal contractile responses to UII could be obtained in $Ca^{2+}$-free buffer solutions. Enzymatically isolated smooth muscle cells from the rat thoracic aorta maintained in primary culture for 3 days could be induced to markedly change their shape in response to UII. Part sequences of the peptide, $UII_{5-12}$ and $UII_{6-12}$ also contracted the aortic strips at $EC_{50}$=$5x10^{-10}$M and $4.1x10^{-9}$M, respectively: the fragment $UII_{5-11}$ had only minimal contractile activity at $10^{-7}$M. Thus, the minimum active core of UII to induce a full contractile response in the rat aortic strips is the ring portion containing the C-terminal amino acid residue.

It may be concluded that the fish neuropeptide UII interacts with vascular smooth muscle cell receptors and induces its effects partly by intracellular $Ca^{2+}$ mobilization but mainly by increased extracellular $Ca^{2+}$ influx, via potential-dependent and potential-independent calcium channels.

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# COMPARISON OF HUMAN AND RAT α-CGRP ON BLOOD PRESSURE AND HEART RATE IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS

R.K. Craig, J.J. Holman*, I. Marshall & N.B. Shepperson[1], Department of Pharmacology, The Middlesex Hospital Medical School, London and [1]Department of Pharmacology, Wyeth Research (UK), Huntercombe Lane South, Taplow, Berkshire.

Calcitonin gene-related peptides (CGRP) lowered the blood pressure of normotensive anaesthetised dogs without increasing heart rate (Craig et al, 1986). The present work compares the hypotensive effect of CGRP in normotensive and hypertensive rats since these differ in their response to some drugs (Ishii et al, 1980).

Sprague-Dawley rats (220-315 g) or Wistar Kyoto derived spontaneously hypertensive rats (SHR, 140-190 g) were anaesthetised with sodium pentobarbitone (60 mg $kg^{-1}$ i.p.). Mean arterial pressure (MAP) and heart rate (HR) were measured via the carotid artery and drugs administered via the jugular vein. Saline or CGRP (Cambridge Research Biochemicals) was given cumulatively at 2 min intervals ($10^{-11}$ - $10^{-8}$ mol $kg^{-1}$). Sodium nitroprusside was given non-cumulatively (1.1 x $10^{-9}$ - 1.1 x $10^{-7}$ mol $kg^{-1}$). The pre-drug MAP of normotensive rats was 137 ± 4 and of SHRs was 185 ± 3 mm Hg. In further experiments the jugular vein and carotid artery of SHRs were cannulated under halothane anaesthesia and exteriorized. 24h later, MAP and HR were recorded from the carotid artery of the conscious rats. Drugs were administered via the jugular vein at 20 min intervals. In these rats the pre-drug MAP was 172 ± 5 mm Hg. Table 1 gives the dose at which the peptides and sodium nitroprusside induce a 25% fall in MAP from the initial value and the maximum percentage fall in MAP, evoked by CGRP, $10^{-8}$ mol $kg^{-1}$ and sodium nitroprusside 1.1 x $10^{-7}$ mol $kg^{-1}$.

Table 1 Effects of CGRP and sodium nitroprusside (SN) on MAP (mean ± s.e. mean)

| | Dose (mol $kg^{-1}$ x $10^{-10}$) giving 25% fall in MAP | | | Maximum % fall in MAP | | |
|---|---|---|---|---|---|---|
| | Anaesthetised | | Conscious | Anaesthetised | | Conscious |
| | Normals | SHRs | SHRs | Normals | SHRs | SHRs |
| h α-CGRP | 2.7 ± 0.4 | 5.0 ± 0.4 | 34 ± 15 | 56 ± 3 | 63 ± 2 | 39 ± 5 |
| r α-CGRP | 2.1 ± 0.2 | 5.9 ± 0.7 | 21.3 ± 3.1 | 52 ± 3 | 61 ± 1 | 36 ± 3 |
| SN | 125 ± 30 | 125 ± 16 | 520 | 59 ± 1 | 60 ± 6 | 31 ± 8 |

In anaesthetised rats the pre-drug HR was 401 ± 10 in normotensives and 365 ± 5 in SHRs. The maximum increase in HR (after $10^{-8}$ mol $kg^{-1}$) was 41 ± 13 and 46 ± 10 $b.min^{-1}$ in normotensives and 45 ± 9 and 38 ± 1 $b.min^{-1}$ in SHRs for human and rat α-CGRP respectively. Sodium nitroprusside did not significantly alter HR. In conscious rats, the pre-drug HR was 410 ± 11 $b.min^{-1}$. In contrast to the results from anaesthetised rats, the maximum increase in HR was 161 ± 13, 156 ± 13 and 104 ± 15 $b.min^{-1}$ for human α-CGRP, rat α-CGRP and sodium nitroprusside respectively.

In conclusion, human and rat α-CGRP were of similar potency as hypotensive agents in anaesthetised normotensive and spontaneously hypertensive rats, but less potent in conscious SHRs. The significantly greater increase in heart rate to CGRP compared with an equi-effective hypotensive dose of sodium nitroprusside is consistent with the direct effect of the peptides in the heart (Al-Kazwini et al, 1985).

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# PROLONGED TREATMENT WITH CAPTOPRIL ATTENUATES PRESSOR RESPONSES TO NORADRENALINE IN THE PITHED SPONTANEOUSLY HYPERTENSIVE RAT (SHR)

J. Atkinson*, A.K. Fouda and M. Sonnay, Institut de Pharmacologie de l'Université, rue du Bugnon 21, 1005 Lausanne, Switzerland.

Although acute administration of captopril, (CAP), a converting enzyme inhibitor, attenuates the pressor response to noradrenaline (NA) (de Jonge et al, 1984; Ubeda et al, 1985), results with prolonged treatment are equivocal (Antonaccio & Kerwin, 1981; Lai et al, 1981). We have reinvestigated whether prolonged treatment with CAP modifies the pressor response to exogenous NA in the pithed SHR.

Three month old, male SHR were fitted with osmotic minipumps (flow rate: 0.5 (group C) or 1 µl/h (group D) and volume 220-240 µl) which delivered CAP (C: 100 mg/ml, D: 250 mg/ml i.v.) or ethanol solvent (group B) i.v. Group A underwent a sham operation in which one external jugular vein was tied off. Group C rats received approximately 4 mg/kg of CAP per day for 17 days, whereas group D rats received 20 mg/kg per day for 4 days. On the 4th or the 17th day, SHR were anesthetized with pentobarbitone and the proximal part of the tail artery removed. Tail arteries were perfused/superfused with Krebs bicarbonate at a constant flow rate of 4 ml/min. They were subject to electrical stimulation (0.1-30 Hz, 0.3 msec, 15 sec, supramaximal voltage) or perfusion with NA (10nM to 3µM). The femoral artery and vein were cannulated (for diastolic arterial pressure (DAP, mmHg) and heart rate (HR, bpm) recording and for administration of NA (0.1nmoles to 10µmoles/kg, i.v.) respectively). Rats were pithed and ventilated at 60 strokes/min at 10 ml/kg per stroke. Dose-response curves to NA were performed and maximal pressor responses (mmHg) and $ED_{50}$s (nmoles/kg) determined.

Results obtained with the tail artery in vitro showed no differences amongst the groups. In vivo there were no differences between sham operated SHR (A) or control (B). The low dose CAP treatment (C) did not alter DAP and HR, the high dose CAP treatment (D) produced a significant fall in DAP. Neither of the CAP treatments altered the maximal pressor response to NA. CAP treatments produced a parallel shift to the right in the NA dose-response curve. The shift was similar in both groups (C: 2.4 fold and D: 2.0 fold).

| | | Before pithing | | After pithing | | Response to NA | |
|---|---|---|---|---|---|---|---|
| Group | n | DAP | HR | DAP | HR | Maximum | $ED_{50}$ |
| A Sham | 6 | 175±8 | 417±25 | 40±2 | 282±8 | 164±13 | 8.5±0.2 |
| B Control/EtOH | 7 | 159±9 | 409±16 | 34±3 | 313±12 | 176±9 | 11.7±2.4 |
| C Low captopril | 4 | 141±11 | 370±27 | 34±4 | 297±18 | 156±8 | 27.7±3.3** |
| D High captopril | 7 | 111±5** | 419±9 | 25±1* | 237±14 | 163±3 | 22.9±1.8** |

Mean ± SEM: statistics: control (B) versus low (C) or high (D) CAP. * $p<0.05$ and ** $p<0.01$ based on the t-test for independent means.

Prolonged treatment with CAP diminishes sensitivity to exogenous NA in SHR. This in vivo effect appears to be independent of the antihypertensive action of CAP as it occurs with both low dose CAP treatment (C), which does not lower DAP and high dose CAP treatment (D), which does lower DAP.

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# FURTHER EVIDENCE FOR THE NEURAL RELEASE OF PLASMA CALCITONIN GENE-RELATED PEPTIDE.

P.J.R. Bevis, I. MacIntyre, M. Zaidi*. Endocrine Unit, Royal Postgraduate Medical School, Hammersmith Hospital, DuCane Road, London W12 OHS. Introduced by J.R. Tippins

We have previously reported that the alternative splice product of the calcitonin (CT) gene, calcitonin gene-related peptide (CGRP) circulates in both man (Girgis et al, 1985) and rat (Zaidi et al, 1986). The peptide is a potent vasodilator in four species including man (Brain et al, 1984) and has potent myotropic properties in the rat (Tippins et al, 1984). We have recently provided evidence for its release into the circulation from perivascular nerves (Zaidi et al, 1985). We now present further evidence for the neural release of CGRP.

CGRP was measured in rat plasma extracts using a specific and sensitive (detection limit 0.25 fmol/assay tube) radioimmunoassay. Using this assay we detected changes in plasma CGRP levels in response to capsaicin (50 mg/kg, s.c., neonatally and/or 10 mg/kg, i.p., as adults) and colchicine (10 mg/kg, i.p., as adults).

Six weeks following neonatal administration of capsaicin, there was a significant ($P < 0.005$) decline of mean plasma CGRP levels. When rats treated with capsaicin at birth were also given capsaicin as adults, there was a six-fold rise of plasma CGRP. In contrast, when control adult rats, not given capsaicin neonatally, were treated with capsaicin as adults, an approximate 13-fold rise of plasma CGRP was observed. In a separate experiment, acute administration of colchicine caused a reduction in peptide levels after 3 hours. Plasma CGRP became undetectable at 6 hours post-treatment. When capsaicin was administered to the group of colchicine treated rats, a less marked rise in plasma CGRP followed.

These results suggest that inhibiting nerve axonal transport of the peptide by colchicine or destroying nerve bodies by neonatal capsaicin treatment causes a significant reduction in circulating CGRP levels. Conversely depolarization of nerve terminals by acute capsaicin treatment of adult rats is followed by a sharp rise of plasma CGRP. This is less obvious in the rats given capsaicin neonatally and again as adults.

These findings confirm that circulating CGRP in rats is predominantly of nervous origin. We also postulate that the predicted physiological role of CGRP on blood flow may be exercised via peptide release from perivascular nerves.

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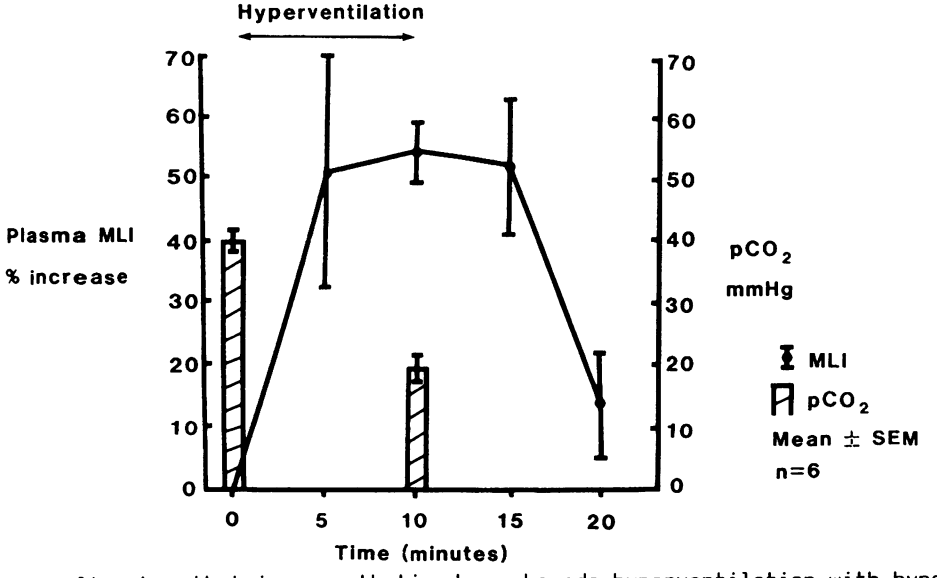
# PLASMA MET-ENKEPHALIN AND CATECHOLAMINES RESPONSES TO ARTIFICIAL HYPERVENTILATION IN ANAESTHETISED GREYHOUNDS

D.F.J. Mason and S. Medbak*, Departments of Pharmacology and Chemical Endocrinology, Medical College of St. Bartholomew's Hospital, Charterhouse Square, London EC1M 6BQ.

The circulating levels of met-enkephalin-like immunoreactivity (MLI) rise following chlorpropamide and ethanol in dogs (1). An infusion of acetaldehyde, the immediate metabolite of ethanol, is also associated with an increase in plasma MLI in greyhounds (1). It was of interest to us that acetaldehyde infusion in dogs provoked hyperventilation. Thus in this study we assessed the plasma MLI responses to hyperventilation in six anaesthetised greyhounds. Anaesthesia was induced by methohexitone and maintained by urethane-chloralose mixture. Blood pressure, heart rate and end expiratory carbon dioxide concentrations were recorded continuously throughout the experiment. Hyperventilation was induced, using room air, by manual compression of an anaesthetic rebreathing bag for 10 minutes. Central venous blood samples were taken before and at regular intervals after the start of hyperventilation for the measurements of plasma MLI, nor-adrenaline and adrenaline concentrations. Arterial blood samples for the measurements of $pCO_2$, $pO_2$ and pH were also taken before and at the end of the period of hyperventilation.

As shown in Fig. 1 there was a marked fall in $pCO_2$ from a basal value of 40.1 ± 1.8 mmHg to 19.7 ± 2.2 mmHg (55.9% ± 5.0% fall; mean ± SEM) after 10 minutes. This was associated with an increase in $pO_2$ (79.0 ± 1.8 mmHg to 100.5 ± 0.6 mmHg) and pH (7.30 ± 0.003 to 7.46 ± 0.003). These changes were associated with a significant increase (over 50%) in plasma MLI (see Fig. 1) which was accompanied by marked elevation in plasma nor-adrenaline (435% increase) and adrenaline (185% increase).




These results show that in anaesthetised greyhounds hyperventilation with hypocapnoea and/or raised oxygen tension will provoke MLI release, together with catecholamines, into the circulation. Whether circulating met-enkephalin is involved in the control mechanisms of respiratory function is not clear at present.

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# LOSS OF RESPONSIVENESS TO ADRENALINE IN IMMUNOLOGICALLY SENSITIZED GUINEA-PIG TRACHEAL MUSCLE.

K.D. Bhoola* & D.M. Crothers, Department of Pharmacology, Medical School, University of Bristol, Bristol, BS8 1TD.

Allergic or extrinsic bronchial asthma is caused by an immediate-type hypersensitivity reaction to allergens, and is primarily mediated by IgE antibodies. Ovalbumin-sensitized guinea-pigs are often used as animal models of extrinsic asthma, even though the main homocytotropic antibody produced is IgG. Depending on the sensitization procedure two types of antibody profile are provoked with ovalbumin. When, IgE antibodies are raised, in addition to IgG the basal activation of guinea-pig lung adenylate cyclase increases two-fold (Bhoola et al 1983; Bhoola & Gadd, 1984b) and the β-adrenoceptor mediated stimulation of the enzyme is significantly reduced (Bhoola & Gadd, 1184b). The molecular change involved, may be a form of uncoupling of the receptor-adenylate cyclase complex, reminiscent of S49 mouse lymphoma cell variants with genetic lesions affecting adenylate cyclase (Johnson et al, 1980). The aim of the present study was to determine whether IgE sensitized lung tracheal muscle would show a similar loss of responsiveness to adrenaline.

Guinea-pigs were sensitized by a single intraperitoneal injection of 1 μg of ovalbumin, suspended in aluminium hydroxide gel (100 mg/ml, dissolved in sterile 0.9% sodium chloride). After a 6 to 7 week interval, the animals were killed by cervical dislocation. Blood was collected from the right ventricle for later analysis of antibody profile. The trachea was removed, placed in modified Krebs-buffer solution and spirally cut to attain an amount of muscle tissue equivalent to that of six tracheal segments. The muscle strip was placed in a 10 ml organ bath containing Krebs-buffer at 37°C and gassed with 95% $O_2$ - 5% $CO_2$. The tracheal muscle strip was tied by a thread to an isotonic transducer with a resting tension of 0.5 gm and the response displayed on a pen recorder.

Dose-response curves were constructed for adrenaline ($3 \times 10^{-8}$M - $2 \times 10^{-6}$M) and theophylline ($2.5 \times 10^{-5}$M - $1.6 \times 10^{-3}$M) on tracheal muscle strips of control and sensitized guinea-pigs and fitted to the logistic expression (Barlow, 1983). The calculated maximum relaxation to adrenaline was significantly less in sensitized guinea-pigs than in controls ($p<0.05$) whereas with theophylline there was no significant difference. Our results provide pharmacological evidence in support of the hypothesis that immunological sensitization linked to the formation of IgE antibodies causes a functional lesion within the β-adrenoceptor -adenylate cyclase complex (Bhoola & Gadd, 1984b).

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# CHARACTERISATION OF β-ADRENOCEPTORS IN GUINEA PIG SKELETAL MUSCLE.

M.S. Elfellah, C.A. Hamilton, J.L. Reid, Department of Materia Medica, Stobhill General Hospital, Glasgow, G21 3UW.

Adrenaline causes hypokalaemia in animals and man (Struthers & Reid, 1984). This response to adrenaline may play an important role in maintaining physiological levels of potassium during and immediately after psychological stress and exercise. In addition the hypokalaemia frequently observed during the early phase of myocardial infarction could be the result of increased plasma adrenaline and may contribute to the genesis of arrhythmias. Stimulation of beta-adrenoceptors mediates the hypokalaemic response to adrenaline. Skeletal muscle is the largest organ in the body that contains potassium, it is likely that beta-adrenoceptors in the muscle are the primary mechanism for the hypokalaemic effect of adrenaline (Clausen & Flatman, 1980). The aim of the present study is to characterise the beta-adrenoceptors in skeletal muscle using radioligand techniques.

Gastrocnemius and soleus muscles from male guinea-pigs weighing 250-350 gm were isolated and tissue membranes prepared as described previously (Karliner et al, 1979) and diluted to approximately 0.4 mg/ml protein. Tissue membrane aliquots of 200 ul each were incubated with (-)-[$^{125}$I] iodocyanopindolol (4-60 pM) in triplicate at 25°C for 90 min in final volume of 400 ul Tris HCl buffer at pH 7.4. Incubations were terminated by vacuum filtration. Specific binding was determined as the difference between binding in the presence and absence of 1 uM(±) propranolol. Inhibition constants ($K_I$) for beta-adrenoceptor agonists and antagonists were determined from competitions curves by the method of Cheng & Prusoff (1973).

The specific binding of the radioligand was saturable (Bmax = 84.0 ± 5.7 and 59.9 ± 8.6 fmole/mg protein, mean ± S.E., for gastrocnemius and soleus muscles respectively), stereospecific for beta-adrenoceptors and with high affinity ($K_d$ = 10.7 ± 1.1 and 11.6 ± 1.4 pM for gastrocnemius and soleus muscles respectively). The $K_I$s for both isomers of propranolol (beta$_1$ and beta$_2$-antagonist) ICI 118551 (beta$_2$-selective antagonist), atenolol and metoprolol (beta$_1$ selective antagonists) are shown in the table and suggest that the receptors are beta$_2$ in type. Hofstee plots for these antagonists were linear indicating that the beta-adrenoceptors in the gastrocnemius muscle are homogenous. Hofstee plot for ICI 118551 was also linear in the soleus muscle.

Table Ki values (nM)(means ± S.E. mean) in gastrocnemius muscles (n=5-14 animals)

| (-)propranolol | (+)propranolol | ICI118551 | atenolol | metoprolol |
|---|---|---|---|---|
| 0.16 (± 0.02) | 19.4 (± 2.2) | 0.39 (± 0.05) | 8381 (± 2063) | 585.0 (± 80.0) |

In conclusion the beta-adrenoceptors in gastrocnemius and soleus muscles from guinea-pigs possess a homogenous population of beta$_2$-adrenoceptors. These results are in agreement with the binding studies in the cat soleus muscle using [$^3$H] dihydroalprenolol as the radioligand (Minneman et al, 1979) and with the hypokalaemia response to beta-adrenoceptor agonists in the rat skeletal muscle (Clausen & Flatman, 1980).

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# EFFECT OF SYMPATHECTOMY UPON $\alpha$- AND $\beta$-ADRENOCEPTOR SENSITIVITY OF THE CAT NICTITATING MEMBRANE IN VIVO

K.J.Broadley , Department of Pharmacology, Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cardiff CF1 3XF

It is suggested that $\beta_1$-adrenoceptors are under the direct influence of noradrenaline released from sympathetic neurones, whereas $\beta_2$-adrenoceptors are stimulated primarily by circulating adrenaline (Ariens & Simonis, 1983). To support this hypothesis, we have shown that responses mediated via $\beta_1$, but not $\beta_2$-adrenoceptors, display supersensitivity after chronic depletion of neuronal catecholamines (Broadley et al., 1984; Hawthorn & Broadley, 1984). The present study examines the effects of unilateral chronic sympathetic decentralization on the in vivo sensitivity of the cat nictitating membrane to the $\alpha$-adrenoceptor-mediated contractile response and the relaxation mediated via $\beta_2$-adrenoceptors (Broadley, 1977). Some $\beta_1$-adrenoceptors are also claimed to exist from in vitro experiments (Varma & Nickerson, 1981a).

Cats (1.7-3.7kg) were anaesthetized with halothane:nitrous oxide and the right superior cervical sympathetic nerve sectioned preganglionically (decentralization). Animals were given streptomycin (0.5g i.m.) and permitted to recover. Six days later they were anaesthetized with chloralose (80mgkg$^{-1}$ i.v.). Trachea, femoral artery and vein and left and right lingual arteries were cannulated. Blood pressure, heart rate and isometric tension of both nictitating membranes were recorded. Drugs were administered either intravenously (i.v.) or close-arterially (i.a.) to the nictitating membranes via the appropriate lingual artery.

The contractile responses to i.v. adrenaline were significantly ($P<0.05$) greater on the right than the left (control) nictitating membrane, the mean contractions (n=4) for the 10$\mu$gkg$^{-1}$ dose being 9.62±0.74g and 4.88±0.63g respectively. ED50 values were not determined, since a maximum contraction of the left side could not be achieved, however, the denervated side was clearly supersensitive to the contractile effects of adrenaline. After phentolamine (8mgkg$^{-1}$ i.v.), adrenaline (i.a.) relaxed the nictitating membranes, but there was no significant ($P>0.05$) difference in sensitivity between right and left sides, the geometric mean (n=4) ED50 values being 8.21 (2.77-24.3) and 6.80 (1.02-45.5)ngkg$^{-1}$ respectively.

The experiments were repeated using noradrenaline and isoprenaline administered i.a. The right nictitating membrane displayed supersensitivity for the contractile responses to noradrenaline. The mean (n=4) ED50 value for the left (control) side (2.97 (0.12-72.5)$\mu$gkg$^{-1}$) was significantly ($P<0.05$) greater than for the right (denervated) side (146.8 (17.8-1207)ngkg$^{-1}$). Isoprenaline relaxed the nictitating membranes but there was no supersensitivity of the denervated side. The mean (n=4) ED50 values when administered to the left (5.12(0.59-44.0)ngkg$^{-1}$) and right side (3.96(1.38-11.4)ngkg$^{-1}$) were not significantly ($P>0.05$) different.

Decentralization of the nictitating membrane therefore results in supersensitivity of the $\alpha$-adrenoceptor-mediated contractile responses, but not of the $\beta_2$-adrenoceptor-mediated relaxation. A similar result has been obtained in isolated nictitating membranes (Varma & Nickerson, 1981b). This supports the contention that $\beta_2$-adrenoceptors are not directly influenced by the sympathetic innervation but are probably stimulated by circulating adrenaline.

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# BRL 24924: A STIMULANT OF GUT MOTILITY WHICH IS ALSO A POTENT ANTAGONIST OF THE BEZOLD-JARISCH REFLEX IN ANAESTHETISED RATS

A.W. Dunbar, C.M. McClelland & G.J. Sanger*, Beecham Pharmaceuticals, Harlow, Essex. CM19 5AD

Novel substituted benzamides, such as BRL 20627 (McClelland et al, 1983) and cisapride (Shuurkes et al, 1985), have been described as weak dopamine antagonists which also increase gut motility, mostly by increasing gut cholinergic activity. Another compound with weak affinity for dopamine receptors is BRL 24924, which is also a potent stimulant of gastric motility in vivo (Cooper et al, 1986) and in vitro (unpublished). We now show that BRL 24924 may also act as a potent antagonist of 5-hydroxytryptamine (5-HT) M-receptor activity, contrasting with the weak 5-HT M-receptor antagonism shown by BRL 20627 or cisapride.

Antagonism of 5-HT M-receptor activity was assessed in vivo using the Bezold-Jarisch reflex (Fozard, 1984). Male Wistar rats (260-290g) were anaesthetised with urethane 1.25g/kg i.p., and the trachea cannulated. Blood pressure was recorded from the left carotid artery, via a saline/heparin-filled pressure transducer from which the heart rate was also continuously monitored. Drugs were injected intravenously (i.v.) into the exterior jugular vein. The Bezold-Jarisch reflex was evoked by rapid, bolus i.v. injection of 5-HT 10-30μg/kg. After obtaining the minimum dose of 5-HT to clearly evoke reflex bradycardia, consistent responses were established every 12 min. Potential 5-HT antagonists were tested by injecting increasing doses, 5 min before each challenge with 5-HT. An $ED_{50}$ was calculated as the dose which reduced the 5-HT-induced bradycardia by 50%; results are given as means ± s.e.m.

BRL 24924 0.3-30-μg/kg i.v. had no effects on resting blood pressure or heart rate, but dose-dependently reduced the Bezold-Jarisch reflex; the $ED_{50}$ was 3.7 ± 1.1μg/kg i.v. (n=8). In contrast, BRL 20627 and cisapride were considerably less active; respective $ED_{50}$ values (mg/kg i.v.) were 0.25 ± 0.1 and 0.24 ± 0.08 (n=4 each). The single dose of BRL 24924 100μg/kg i.v., produced a long-lasting reduction of the Bezold-Jarisch reflex, which was reduced by 94 ± 5% and by 71 ± 7% respectively, 5 and 89 min after injection of BRL 24924 (n=10).

In some experiments, bradycardia was evoked by electrical stimulation of the peripheral end of a cut vagus nerve, the efferent limb of the Bezold-Jarisch reflex. Stimulation was applied every 5 min via a pair of silver electrodes, using 1ms pulses in 5s trains, with a maximum-effective voltage (20V at 10Hz). Pulse frequency varied from 5-30Hz and frequency-response curves were constructed before and 10 min after injection of BRL 24924 100μg/kg i.v. Compared with the first frequency response curve, BRL 24924 did not affect ($P>0.1$, Students t-test; n=4) the bradycardia evoked by each frequency of stimulation. Antagonism by BRL 24924 of the Bezold-Jarisch reflex may therefore be unrelated to a change in efferent activity.

BRL 24924 is therefore a compound with a unique profile of activity. Unlike BRL 20627 and cisapride, BRL 24924 potently inhibits the Bezold-Jarisch reflex in anaesthetised rats and may therefore act as a 5-HT M-receptor antagonist. In addition, BRL 24924 is a highly potent stimulant of gut motility with poor affinity for dopamine receptors (Cooper et al, 1986).

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# OXYGEN MODIFIES THE POTENCY OF 5-HT, AND OF 5-HT ANTAGONISTS, IN HUMAN UMBILICAL ARTERY (HUA)

J.C. McGrath and S.J. MacLennan*. Institute of Physiology, The University, Glasgow G12 8QQ.

We have shown that 5-HT receptors in HUA are competitively antagonised by methysergide and phentolamine (McGrath et al, 1985). We now employ the 5-$HT_2$ antagonist ketanserin to further characterise the receptors and demonstrate different populations according to the $pO_2$.

Umbilical cords collected after birth were stored in ice-cold Krebs-bicarbonate (4°C) for up to 24 hours. Isometric tension was recorded from longitudinal strips of artery in Krebs at 37°C. To mimic physiological blood gas tensions, in utero, the Krebs was bubbled with 2.5% $O_2$, 8% $CO_2$, balance $N_2$ which gave: $pO_2$ 15mmHg, $pCO_2$ 48mmHg, pH 7.28. $pO_2$ was monitored continuously using an IL1302 $O_2$ electrode. Cumulative concentration-response curves to 5-HT were constructed and repeated after 1h in the presence of graded concentrations of antagonist in different strips of artery, or after changing the gas mixture.

At physiological $pO_2$ (15mmHg) 5-HT contracted HUA over the range 10nM-10uM ($pD_2$ 7.24±0.11), (mean±s.e.mean). Indomethacin (1uM) did not antagonise 5-HT but the maximal response was significantly ($P<0.05$) increased (118±4% of control maximum). Paired control tissues showed a smaller but significant increase of the maximum (104±3% of first maximum). At $pO_2$ 120mmHg the potency of 5-HT was significantly greater ($pD_2$ 7.75±0.08). Indomethacin (1uM) abolished this increased potency.

At $pO_2$ 15mmHg ketanserin (1nM-0.1uM) produced a concentration related parallel shift to the right of the upper part of the 5-HT concentration-response curve ($pA_2$ assessed from the shift in $EC_{50}$ =8.92). However the shifts were not parallel at low concentrations of 5-HT. In the presence of ketanserin (0.1uM) the shifts at $EC_{25}$ and $EC_{50}$ were 89 and 133 fold respectively. At $pO_2$=120mmHg ketanserin (0.1uM) caused a distinctively biphasic antagonism. Lower concentrations of 5-HT were poorly antagonised by ketanserin (0.1uM); the shift at the $EC_{25}$ was 4 fold and at $EC_{50}$ was 121 fold. In the presence of indomethacin (1uM), ketanserin (0.1uM) caused a parallel shift of the 5-HT curve at all concentrations; the $EC_{25}$ and $EC_{50}$ were shifted by 444 and 895 fold respectively.

Methysergide, at $pO_2$=15mmHg, showed no agonism. At $pO_2$=120mmHg methysergide (1 or 0.1uM) induced rhythmic contractions of varying amplitude and frequency, hence complicating the analysis of its antagonism at this higher $pO_2$. The maximum tone induced by methysergide was taken as the baseline tension for analysis of 5-HT's effect. Methysergide (0.1uM) caused a biphasic shift of the 5-HT curve. At lower concentrations of 5-HT the curve in the presence of methysergide showed a parallel shift with an inflection in the curve at concentrations (of 5-HT) producing 50% of the maximum response. After incubation with indomethacin (1uM), methysergide caused a parallel displacement of the 5-HT curve at all concentrations.

At $pO_2$=120mmHg, phentolamine (100uM) had no agonism and caused a parallel displacement of the 5-HT curve, in the presence or absence of indomethacin (1uM).

These results suggest that two receptors for 5-HT may exist in HUA. (1) At physiological $pO_2$ or at high $pO_2$ in the presence of indomethacin, ketanserin, methysergide and phentolamine act as competitive antagonists; presumably this is 5-$HT_2$. At higher $pO_2$'s a ketanserin resistant but phentolamine and indomethacin-sensitive receptor is present. Human saphenous vein also has a ketanserin-resistant component to 5-HT (Docherty & Hyland, 1985). Therefore, 5-HT receptors exist in human tissues, in addition to 5-$HT_2$, which do not fit into current categories for 5-HT receptor sub-types and, in HUA, have a prostaglandin involvement.

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# MEPTZINOL-INDUCED DIURESIS IN THE WATER-LOADED RAT.

D.R. Algate, N.T. Brammer and D. Lawrence*, Department of Pharmacology, Huntingdon Research Centre Limited, Huntingdon, Cambridgeshire, PE18 6ES.

Opioid agonists acting at the kappa receptor induce diuresis in normally hydrated or water-loaded rats, whereas mu receptor agonists produce antidiuresis (Leander 1982, Skingle et al. 1985). In this study the effect of the opioid agonist meptazinol on urine output was examined in the water-loaded rat. A minimum of 5 female Wistar rats (200 - 250 g) were treated subcutaneously with the test compound, water-loaded at a dose volume of 25 ml/kg and placed individually in metabolism cages. The cumulative urine output was measured hourly over a 5 hour period.

Following treatment with meptazinol there was a dose-related increase in urine output. Meptazinol was less active than the kappa agonist cyclazocine but displayed no antidiuretic effect as seen with morphine.

Table 1. The effect of meptazinol, cyclazocine and morphine on urine output in the water-loaded rat.

| Treatment | Dose (mg/kg) | Percentage difference from control urine output 0 - 2 hr | 0 - 5 hr |
|---|---|---|---|
| Meptazinol | 30 | + 84.4% | + 69.1% |
| Cyclazocine | 3 | + 211.0% | + 113.3% |
| Morphine | 10 | - 65.0% | - 15.4% |

The cumulative urine output of control animals was between 2.7 - 5.3 ml 2 hours post-dose and between 4.2 - 7.0 ml 5 hours post-dose. Subcutaneous administration of the opiate antagonist naloxone at 0.1, 1.0 and 10.0 mg/kg inhibited the meptazinol (30 mg/kg)-induced diuresis by 24.8%, 66.4% and 77.9% respectively at 5 hours post-dose. In comparison, cyclazocine (3 mg/kg)-induced diuresis was reduced by 0%, 14.1% and 39.8% by the same dose-levels of naloxone. The reportedly more potent kappa antagonist MR-2266 (Romer et al. 1980), at dose levels of 1 and 3 mg/kg reduced the meptazinol-induced diuresis by 11.8% and 19.7% respectively. While reductions of 9.8% and 32% in the cyclazocine-induced diuresis were observed following MR-2266 at the same dose levels.

These results indicate that meptazinol does not appear to have any antidiuretic effects but increases urine output in the water-loaded rat, an effect possibly mediated at the kappa opiate receptor. Receptor binding data, however, indicates meptazinol has $mu_1$ receptor selectivity (Green, 1983).

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# $\alpha_2$-ADRENOCEPTOR REGULATION OF ELECTROLYTE TRANSPORT IN RAT JEJUNUM

P. W. Dettmar[1], O. A. Downing, A. G. Roach[1], R. J. Williams* & K. A. Wilson, Pharmaceutical Sciences Institute, Aston University, Birmingham, B4 7ET and [1] Department of Pharmacology, Reckitt & Colman plc, Hull HU8 7DS

Specific $\alpha_2$-adrenoceptor stimulation has been shown to reverse secretagogue induced fluid secretion in rat jejunum in vivo (Nakaki et al, 1982; Bunce & Spraggs, 1983). Since changes in fluid absorption or secretion in vivo may result from changes in intestinal blood flow, we considered it worthwhile to investigate the role of $\alpha$-adrenoceptors in the control of electrolyte transport in vitro using a modified Ussing chamber.

Tissues were obtained from male Wistar rats (160-200g) anaesthetised with sodium pentobarbitone. Measurements were made of the changes in resting short circuit current (SCC) induced by noradrenaline (NA), phenylephrine (PE) or UK14304 (UK), alone or in the presence of a number of $\alpha$-adrenoceptor antagonists. Resting SCC was stable 20-30 minutes after mounting the tissues. NA ($5 \times 10^{-6}$) and UK ($10^{-7}$M) caused an immediate, sustained fall in SCC which was maximal within 3 minutes whereas only a high dose of PE ($10^{-4}$M) produced this effect. These changes in SCC were significantly greater than changes in control tissues over the same time period, ($-1 \pm 1$ $\mu$amp.cm.$^{-1}$; $p<0.001$). The results in table 1 show that the fall in SCC induced by NA was significantly inhibited by phentolamine ($5 \times 10^{-6}$M), idazoxan ($10^{-6}$)M, and yohimbine ($5 \times 10^{-6}$M), but not by prazosin ($2.5 \times 10^{-5}$M) or corynanthine ($10^{-4}$M). None of these antagonists alone caused any significant change in SCC ($p>0.05$). However, propranolol did reduce resting SCC by $14 \pm 3$ $\mu$amps.cm$^{-1}$ ($n=5$) although NA still caused a significant fall in SCC of $12 \pm 2$ $\mu$amp cm$^{-1}$ in the presence of propranolol ($p<0.05$). The change in SCC observed with UK ($10^{-7}$M) was inhibited by idazoxan ($5 \times 10^{-6}$M) but not by corynanthine ($10^{-4}$M). Similarly the fall in SCC in response to PE ($10^{-4}$M) was inhibited by idazoxan ($5 \times 10^{-7}$M), but not by corynanthine ($10^{-4}$M). After addition of idazoxan ($5 \times 10^{-6}$M), PE ($10^{-4}$M), induced a transient increase in SCC which was inhibited by prazosin.

Table 1. Changes in SCC induced by NA ($5 \times 10^{-6}$M), PE ($10^{-4}$M) and UK ($10^{-7}$M)

| Agonist | Antagonist (max. concn. used) | SCC $\mu$amps.cm$^{-1}$ | P* (n= 5) NS:p>0.05 |
|---|---|---|---|
| NA | - | -24 ± 4 | - |
| NA | Phentolamine ($5 \times 10^{-6}$M) | -2 ± 2 | <0.01 |
| NA | Idazoxan ($10^{-6}$M) | +1 ± 1 | <0.001 |
| NA | Yohimbine ($5 \times 10^{-6}$M) | 0 ± 3 | <0.01 |
| NA | Corynanthine ($10^{-4}$M) | -16 ± 2 | NS |
| NA | Prazosin ($2.5 \times 10^{-5}$M) | -17 ± 3 | NS |
| PE | - | -17 ± 3 | - |
| PE | Idazoxan ($5 \times 10^{-7}$M) | +18 ± 3 | <0.001 |
| PE | Corynanthine ($10^{-4}$M) | -13 ± 2 | NS |
| UK14304 | - | -18 ± 1 | - |
| UK14304 | Idazoxan ($5 \times 10^{-6}$M) | -3 ± 1 | <0.001 |
| UK14304 | Corynanthine ($10^{-4}$M) | -15 ± 2 | NS |

*Students' t-test for unpaired data, comparisons with agonist response alone.

These results provide evidence for an $\alpha_2$-adrenoceptor mediated effect on electrolyte transport in the rat jejunum which probably reflects a depression of anion secretion, but do not preclude the possiblility of an $\alpha_1$-adrenoceptor effect upon absorptive processes.

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