ANXIOGENIC AGENTS INCREASE TRIBULIN CONCENTRATIONS IN RAT BRAIN AND HEART

S.K. Bhattacharya, A. Clow*, V. Glover and M. Sandler, Department of Chemical Pathology, Queen Charlotte's Hospital, London W6 OXG.

We have considerable evidence that increased amounts of an endogenous monoamine oxidase inhibitor, which we have called tribulin (Sandler, 1982), are generated in conditions of stress and anxiety. In this study we have investigated the effects of the anxiogenic drugs pentylenetetrazole (PTZ) and ephedrine on tribulin levels in rat brain and heart.

Female Wistar rats (approximately 250g) were injected (i.p.) with PTZ (20mg/kg), ephedrine (20mg/kg) or water, 30 min prior to death, or diazepam (1 and 2.5mg/kg) or propranolol (5mg/kg) 60 min before death. When two drugs were administered, a 30 min preadministration period was employed. Rat brains and hearts were rapidly removed, weighed and homogenised (20% w/v) in cold 2M HCl, using an Ultraturrax homogenizer. Homogenates were centrifuged for 10 min at 3,000g and the supernatant extracted into two volumes of redistilled ethyl acetate. The organic layer was removed and dried under nitrogen. Acid blanks were run in parallel throughout the procedure. Residues were reconstituted in 100µl of 100mM phosphate buffer, pH 7.4 and incubated with 20µl MAO preparation (1% w/v rat liver homogenate) and 10µl ^{14}C-tyramine (New England Nuclear Corporation); diluted with unlabelled tyramine to give a final concentration of 83µM for 30 min at 37^{0}C.

Table 1 Effect of drugs on endogenous MAO inhibitor level in rat brain and heart (mean ± SEM)

Dose mg/kg i.p.	Drug	N	% Inhibition of MAO Brain	Heart
-	Control	18	26.8 ± 2.3	42.9 ± 2.4
20	PTZ	12	44.9 ± 2.4**	65.1 ± 1.7**
1	Diazepam	9	21.6 ± 3.6	45.6 ± 3.6
2.5	Diazepam	6	24.9 ± 2.6	29.3 ± 5.1*
20 + 1	PTZ + diazepam	9	40.6 ± 3.6*	53.4 ± 4.8*
20 + 2.5	PTZ + diazepam	6	23.3 ± 4.9	29.8 ± 5.9*
20	Ephedrine	6	38.1 ± 2.3**	44.2 ± 2.4
5	Propranolol	6	27.0 ± 1.7	35.6 ± 3.1
20 + 5	Ephedrine + propranolol	6	24.5 ± 1.4	36.7 ± 1.7

Different from control: *$p<0.05$, **$p<0.001$ - Student's t test

It will be seen (Table 1) that anxiogenic doses of PTZ caused a substantial increase in endogenous MAO inhibitory activity in rat brain. Diazepam, which had no intrinsic activity, was able to prevent this. Similarly, ephedrine administration brought about raised MAO inhibitory activity in rat brain which could be prevented by prior administration of propranolol. Similar results were found in the heart, although ephedrine did not cause increased MAO I in the heart.

We have shown previously that rat urinary tribulin is increased by cold restraint stress, an effect attenuated by benzodiazepines (Glover et al., 1981). These results show that anxiogenic agents can also increase levels of this endogenous MAO inhibitor in rat brain and heart, an effect similarly attenuated by anxiolytic agents.

Sandler, M. (1982) Trends Pharmac.Sci., 471-472.
Glover, V., Bhattacharya, S.K., Sandler, M. & File, S. (1981) Nature, 292, 347-349.

2P

THE PHARMACOLOGICAL PROPERTIES OF MDL 73005EF: A POTENT AND SELECTIVE LIGAND AT 5-HT_{1A} RECEPTORS

M. Hibert, A.K. Mir , G. Maghioros, P. Moser, D.N. Middlemiss, M.D. Tricklebank and J.R. Fozard, Merrell Dow Research Institute, 16 Rue D'Ankara, 67084 Strasbourg, France

MDL 73005EF (8-[2-[(2,3-dihydro-1,4-benzodioxin-2-yl)methylamino]ethyl]-8-azaspiro [4,5]decan-7,9-dione methane sulphonate) evolved from a programme aimed at the rational design of 5-HT_{1A} receptor ligands using graphics computer assisted technology (Hibert et al., 1986). This report describes the characterisation of MDL 73005EF as a potent and highly selective ligand for 5-HT_{1A} receptors and its activity in a variety of in vitro and in vivo test systems.

In a variety of radioligand binding assays (for details see Fozard et al., 1987) MDL 73005EF proved a highly selective displacer of binding to the 5-HT_{1A} recognition site (Table 1).

Table 1 Affinity of MDL 73005EF for central neurotransmitter recognition sites ($pIC_{50} \pm SEM$, n = 3 - 6)

5-HT_{1A}	5-HT_{1B}	5-HT_{2}	α_1	α_2	D_1	D_2	β	BDZ
8.6 ± 0.1	4.5 ± 0.3	5.5 ± 0.1	5.6 ± 0.1	5.4 ± 0.1	4.3 ± 0.2	6.4 ± 0.1	4.6 ± 0.1	< 4

In functional tests the agonist and antagonist activity of MDL 73005EF has been evaluated by its ability to either mimic or reverse the effects of the selective 5-HT_{1A} receptor agonist 8-hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT). On the transmurally stimulated guinea-pig ileum (Fozard and Kilbinger, 1985), MDL 73005EF blocked the 8-OH-DPAT-induced inhibition of the contractile response with a -log K_B value of 8.5. The compound was a weak inhibitor of the contractile response (pIC_{15}, 6.7) but achieved only around 50% of the maximum response to 8-OH-DPAT. In anaesthetized normotensive rats MDL 73005EF antagonised the bradycardia and hypotensive effects of a submaximal dose of 8-OH-DPAT (32 µg/kg i.v.; Fozard et al., 1987) in a dose-dependent manner, with complete blockade being achieved at 3 mg/kg s.c. and 5 mg/kg p.o. At these doses MDL 73005EF had minimal effects on BP and HR per se. In male Sprague Dawley rats treated with reserpine, 1 mg/kg 18 h prior to testing, 8-OH-DPAT, 0.125 mg/kg s.c., induces forepaw treading (FPT) and flat body posture (FBP) (Tricklebank et al., 1985). In the same model MDL 73005EF, 0.125 - 8 mg/kg s.c., had minimal agonist effects, whereas over the same dose range the compound inhibited dose-dependently the 8-OH-DPAT-induced FPT. In animals trained to discriminate 8-OH-DPAT from saline in a drug discrimination paradigm (Tricklebank et al., 1987), MDL 73005EF, 0.3 - 3 mg/kg s.c., did not antagonise the 8-OH-DPAT cue; rather, the compound generalised dose-dependently and completely (at 3 mg/kg s.c.) to the 8-OH-DPAT cue.

These results characterize MDL 73005EF as a potent and highly selective ligand at central 5-HT_{1A} recognition sites. With the exception of the drug discrimination paradigm, its functional interactions are manifested predominantly as antagonism. A reduction in serotoninergic tone by blockade of 5-HT_{1A} receptors could be relevant to the activity of MDL 73005EF in animal models of anxiety (Moser et al., this meeting).

Fozard, J.R. and Kilbinger, H. (1985) Brit. J. Pharmacol. 86, 601P.
Fozard, J.R. et al. (1987) J. Cardiovasc. Pharmacol. 9, 328 - 347.
Hibert, M. et al. (1986) J. Mol. Graphics 4, 227P.
Tricklebank, M.D. et al. (1985) Eur. J. Pharmacol. 106, 271 - 282
Tricklebank, M.D. et al. (1987) Eur. J. Pharmacol. 133, 47 - 56.

EFFECTS OF MDL 73005EF IN ANIMAL MODELS PREDICTIVE OF ANXIOLYTIC ACTIVITY

P. Moser*, M. Hibert, D.N. Middlemiss, A.K. Mir, M.D. Tricklebank and J.R. Fozard, Merrell Dow Research Institute, 16 Rue D'Ankara, 67084 Strasbourg, France

MDL 73005EF has been shown to be a potent and selective ligand for the 5-HT_{1A} receptor and in most pharmacological tests for activity at this site it acts primarily as an antagonist (Hibert et al., this meeting). This is unlike most of the compounds currently available which are either full agonists (8-OH-DPAT) or partial agonists with agonist activity predominating, such as buspirone. In view of the evidence linking a decrease in 5-HT function with an anxiolytic action it was of interest to compare the activity of MDL 73005EF with 8-OH-DPAT and buspirone in animal models predictive of anxiolytic activity.

A punished drinking procedure similar to that described by Vogel et al. (1971) was carried out using male Sprague Dawley rats (170 - 210 g). They were deprived of drinking water for 48 h and pretested on the morning of the experiment to exclude those that did not find the punishment aversive (0.4 mA shocks for 1 s delivered via the drinking spout every 20 licks) or those that found it too aversive. Following the pre-selection the rats were assigned to treatment groups for the experimental session in the afternoon. The test session lasted 3 min and the number of licks made under the same punishment schedule as used in the pretest were recorded. Diazepam (0.125 - 2 mg/kg s.c.), MDL 73005EF (0.3 - 3 mg/kg s.c.) and buspirone (0.25 - 2 mg/kg s.c.) given 30 min prior to testing dose-dependently increased the amount of shock-punished licking. MDL 73005EF given orally (1.25 - 20 mg/kg, 15 min pretreatment) also dose-dependently increased the number of shocks accepted. The involvement of the 5-HT_{1A} receptor in this response was supported by the dose-related antagonism of the effect of MDL 73005EF (10 mg/kg p.o.) by 8-OH-DPAT given 15 min prior to testing over the range of 30 - 150 µg/kg s.c. In contrast, 8-OH-DPAT did not significantly inhibit the effects of buspirone (10 mg/kg p.o.). 8-OH-DPAT itself had no significant effect on punished drinking.

The effects of MDL 73005EF were also studied in male Sprague Dawley rats (250 - 300 g) using the elevated plus-maze test of Handley and Mithani (1984) except that the test was carried out in a darkened room and all observations were made via a closed circuit TV camera. Over the dose range 0.03 - 0.25 mg/kg s.c. and 0.25 - 1 mg/kg p.o. MDL 73005EF caused a dose related increase in open arm entries (vehicle: 12.2 $\pm$ 2.9%, MDL 73005EF 0.25 mg/kg s.c.: 31.6 $\pm$ 3.6%: $p < 0.01$ Mann-Whitney U-test). Diazepam also increased the percentage of open arm entries over the range 0.25 - 2 mg/kg s.c. (vehicle: 7.8 $\pm$ 2.9%, diazepam 1 mg/kg: 33.8 $\pm$ 4.3%, $p < 0.01$). At higher doses of MDL 73005EF both the percentage of open arms entered and the total number of arms entered decreased. Similar decreases were also seen with 8-OH-DPAT (12.5 - 100 µg/kg s.c.) and with buspirone (0.125 - 2 mg/kg s.c.).

The effects of MDL 73005EF in these tests indicate putative anxiolytic activity. Since 8-OH-DPAT showed no anxiolytic effects, it seems likely that it is the 5-HT_{1A} antagonist activity of MDL 73005EF that is relevant to its effects in these tests. The difference between MDL 73005EF and buspirone in the plus-maze may reflect the predominantly agonist actions of the latter.

Handley, S.L. and Mithani, S. (1984) Naunyn-Schmiedeberg's Arch. Pharmacol. 327, 1 - 5

Vogel, J.R. et al. (1971) Psychopharmacologia 21, 1 - 7

4P

IN VIVO INTERACTION OF ZOLPIDEM WITH CENTRAL BENZODIAZEPINE BINDING SITES IN THE MOUSE BRAIN

B. Scatton*, J. Benavides, B. Peny, A. Dubois, G. Perrault, E. Morel, B. Zivkovic. Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.) 31 ave P. Vaillant Couturier, 92220 - Bagneux, France.

Zolpidem is a novel hypnotic with rapid onset and short duration of action which possesses weak myorelaxant and anticonvulsant activities (Arbilla et al, 1985; Depoortere et al, 1986; Nicholson et al, 1986). Zolpidem has been shown to possess preferential affinity under in vitro conditions for the BZD_1 ($\omega 1$ according to the new nomenclature proposed by Langer and Arbilla, 1987) subtype of benzodiazepine recognition sites (Arbilla et al, 1985). In the present study, we have investigated the in vivo interaction of zolpidem with mouse brain benzodiazepine binding sites, as labelled by i.v. injection of ^{3}H-Ro 15-1788 (2.5 μCi).

Intraperitoneal administration of zolpidem (30 min before sacrifice) decreased in a dose-dependent manner, the retention of ^{3}H-Ro 15-1788 in the cerebral cortex (ED_{50} = 8.9 mg/kg i.p.); the inhibition by zolpidem was short lasting being maximal (70 %) at 5-10 min post-injection and reduced to only 20 % 1 h later. CGS 9896, CL 218,872 and flunitrazepam also prevented the cortical accumulation of ^{3}H-Ro 15-1788 with ED_{50}'s of 12.5, 24 and 0.17 mg/kg i.p., respectively. The comparison of the ED_{50} values for the anticonvulsant (pentetrazole and electroshock), sedative and myorelaxant effects of zolpidem with the degree of occupancy of the benzodiazepine binding sites by this compound in vivo revealed that a 50 % decrease in exploratory activity required only a 35 % occupation of the benzodiazepine binding sites by zolpidem, whereas a higher degree of occupancy (50-56 %) was necessary for inducing the myorelaxant and anticonvulsant effects.

We have also assessed the regional selectivity of zolpidem as a displacer of ^{3}H-Ro 15-1788 binding in the mouse brain by quantitative autoradiography. In vitro, zolpidem was a more potent displacer of ^{3}H-Ro 15-1788 in BZD_1 ($\omega 1$) enriched regions e.g. molecular layer of the cerebellum, inferior colliculus, substantia nigra, layer IV of the cerebral cortex (Ki at 37°C, 11-12 nM and at 4°C, 10-16 nM) than in BZD_2 ($\omega 2$) enriched regions e.g. superficial layer of superior colliculus, hippocampus, dentate gyrus, striatum (Ki at 37°C, 42-107 nM; at 4°C, 69-112 nM). Similarly, in vivo, zolpidem was more active in preventing ^{3}H-Ro 15-1788 binding in BZD_1 ($\omega 1$) enriched structures (ED_{50}'s 3.6 to 6.7 mg/kg i.p. than in BZD_2 ($\omega 2$) enriched regions ($ED_{50} > 30$ mg/kg i.p.). These data indicate that zolpidem preferentially interacts in vivo with BZD_1 ($\omega 1$) benzodiazepine recognition sites and that BZD_1 ($\omega 1$) and BZD_2 ($\omega 2$) subtypes represent two pharmacologically distinct entities in the in vivo environment.

Arbilla, S. et al (1985) Naunyn Schmiedeberg's Arch. Pharmacol. 330, 248.

Depoortere, H. et al (1986) J. Pharmacol. Exp. Ther. 237, 649.

Langer, S.Z. and Arbilla, S. (1987) 5th International Congress of Sleep Research, Copenhagen.

Nicholson, A.N. and Pascoe, P.A. (1986) Brit. J. Clin. Pharmacol. 21, 205.

AUTORADIOGRAPHIC ANALYSIS OF BENZODIAZEPINE RECEPTOR BINDING IN RAT STRIATUM AFTER DECORTICATION PLUS DIAZEPAM ADMINISTRATION

R. Moratalla*, T.M. Barth & N.G. Bowery. Department of Pharmacology, School of Pharmacy, 29/39 Brunswick Square, London & Neuroscience Research Centre, MSD Terlings Park, Harlow, Essex.

It has previously been shown that diazepam impairs behavioural recovery from partial unilateral cerebral cortex ablation (Schallert et al 1986). Rats treated with diazepam 5 mg/kg i.p. (3 times/day for 3 days followed by 2 injections/day for 19 days) failed to show any recovery from an impairment of sensory assymetry even after 84 days whereas saline-treated animals recovered completely after 12 days. We have now examined whether the lack of recovery after diazepam administration is associated with any long term changes in benzodiazepine receptor binding on the decorticated side. Benzodiazepine receptors were labelled with [^{3}H]Ro15-1788, the benzodiazepine antagonist, and the binding assessed by receptor autoradiography. Our studies so far have been confined to the striatum.

Rats were anaesthetized with equithesin (0.3ml/100g) and a piece of skull removed from the right hemisphere 4mm anterior and 2mm posterior to bregma. The incision extended from the midline to the edge of the skull. Cerebral cortex tissue was aspirated from the brain, care being taken not to damage the underlying striatum. Rat were injected with diazepam (5 mg/kg) as described by Schallert et al (1986). 7,14,30,60 & 120 days after surgery the sensory assymetry was measured as described by Schallert et al (1986).

At each of these periods, 3 test and 3 saline injected rats were perfused-fixed with 0.1% paraformaldehyde and transverse brain sections (10 µm) prepared for autoradiography studies. [^{3}H]Ro15-1788 binding to these sections was performed according to the method of Richards et al (1986). Labelled sections were juxtaposed to Hyperfilm and the resulting images analysed using a Quantimet 970 analyser.

As previously reported diazepam treatment prevented recovery from sensory assymetry even after 120 days whereas after saline administration >80% recovery occurred. Binding of [^{3}H]Ro15-1788 in the caudate putamen on the decorticated side of untreated animals was increased at all time intervals between 14 and 60 days. The mean increases were 25%, 29% and 30% respectively at 14, 30 & 60 days (n>50 sections at each time). The binding of [^{3}H]Ro15-1788 was also increased on the decorticated side after diazepam treatment (24%, 26% 38%, 43% at 14, 30, 60 & 120 days respectively). These values were not significantly different from those obtained in untreated rats.
Binding of [^{3}H]Ro15-1788 in the nucleus accumbens was not altered by decortication alone or after diazepam treatment.

In conclusion, decortication increased the binding of [^{3}H]Ro15-1788 in the caudate putamen but not in the nucleus accumbens. This increase was not affected by chronic diazepam treatment. Thus the lack of recovery from sensory assymetry produced by diazepam after decortication could not be correlated with any change in benzodiazepine receptor binding within the striatum.

Richards, J.G., Möhler, H. & Haefely, W. (1986). In Neurohistochemistry : modern methods and application. Eds. P. Panulla, H. Paivarinta & S. Sonila Publ. Alan. R. Liss, New York. pp. 629-677.
Schallert, T., Hernandez, T.D. & Barth, T.M. (1986). Brain Res., 379, 104-111.

6P

EFFECTS OF Ca^{2+} ON [^{3}H]DILTIAZEM BINDING TO THE RAT CEREBRAL CORTEX

H. Schoemaker*& S.Z. Langer, Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France.

Multiple recognition sites associated with the slow voltage-dependent calcium channel have been identified for dihydropyridine-type (e.g. [^{3}H]nitrendipine), phenylalkylamine-type (e.g. [^{3}H]verapamil) and benzothiazepine-type (e.g. [^{3}H]diltiazem) calcium channel antagonists. In addition, mutual allosteric interactions, in part dependent on the incubation temperature, between these calcium channel antagonist recognition sites have been characterized. Thus, verapamil allosterically inhibits [^{3}H]nitrendipine binding, whereas diltiazem inhibits [^{3}H]nitrendipine binding at 0°C but stimulates [^{3}H]nitrendipine binding at 37°C. In view of the association of calcium channel antagonist recognition sites with the calcium channel, we evaluated the effects of Ca^{2+} on [^{3}H]diltiazem and [^{3}H]nitrendipine binding.

The cerebral cortex of male Sprague-Dawley rats was homogenized in 20 volumes of 50 mM Tris-HCl buffer and washed three times by centrifugation (49000 g, 10 min) and resuspension of the resulting pellet. [^{3}H]Diltiazem binding (4 nM; spec. act. 72 Ci/mmol, New England Nuclear) was measured by incubation for 60 min at 37°C or 180 min at 0°C (Schoemaker and Langer, 1985). The binding of [^{3}H]nitrendipine (0.1 nM; spec. act. 70 Ci/mmol, New England Nuclear) was measured over 30 min at 37°C. Following incubation, membranes were harvested by filtration over Whatman GF/B filters, pretreated with 0.05% polyethylimine in the case of [^{3}H]diltiazem binding, and washed with three 5 ml volumes of ice-cold buffer. Specific [^{3}H]diltiazem binding was defined using 10 µM unlabelled diltiazem and that of [^{3}H]nitrendipine using 1 µM nifedipine.

Whereas at 37°C Ca^{2+} ions do not significantly affect [^{3}H]nitrendipine binding to the rat cerebral cortex up to concentrations of 10 mM $CaCl_2$, [^{3}H]diltiazem binding is inhibited by Ca^{2+} with an IC_{50} of 314 $\pm$ 42 µM (n=5). Ca^{2+}, in concentrations from 10 µM to 10 mM $CaCl_2$, causes a parallel right-ward shift in the concentration-effect curve for the stimulation of [^{3}H]nitrendipine binding by diltiazem with a two-fold shift being observed at 230 µM $CaCl_2$. Thus, in the absence of $CaCl_2$, diltiazem enhances [^{3}H]nitrendipine binding with half-maximal enhancement being observed at a concentration (EC_{50}) of 0.270 $\pm$ 0.050 µM diltiazem, but in the presence of 10 mM $CaCl_2$ its EC_{50} is significantly ($p < 0.02$) increased to 3.30 $\pm$ 0.39 µM. Maximal stimulation of [^{3}H]nitrendipine binding by diltiazem, however, is not affected by Ca^{2+}. Similarly, Ca^{2+} causes a parallel displacement to the right of the allosteric inhibition of [^{3}H]nitrendipine binding by verapamil (IC_{50} at 0 and 10 mM $CaCl_2$: 0.067 $\pm$ 0.006 and 0.729 $\pm$ 0.041 µM).

The present data indicate that [^{3}H]diltiazem binding to the calcium channel is sensitive to inhibition by Ca^{2+} to a significantly greater extent than that of [^{3}H]nitrendipine. At the level of its allosteric modulation of [^{3}H]nitrendipine binding, Ca^{2+} affects the EC_{50} of diltiazem but not its maximal effect.

Schoemaker, H. & Langer, S.Z. (1985) Eur. J. Pharmacol. 111: 273-277.

DIFFERENTIAL INTERACTIONS BETWEEN BENZODIAZEPINES AND DIHYDROPYRIDINES

S. J. Dolin* & H. J. Little[1]. Department of Clinical Pharmacology, Addenbrooks Hospital, Hills Road, Cambridge and [1]Department of Pharmacology, The Medical School, University Walk, Bristol, BS8 1TD

Whilst the majority of the effects of benzodiazepines are thought to be mediated through the classical type of benzodiazepine receptor, this may not be so for all actions. We have shown previously that their general anaesthetic properties were not antagonised by the specific antagonist Ro 15-1788 (Little & Bichard, 1984), suggesting the involvement of another mechanism. We have also reported that the general anaesthetic actions of several agents, including ethanol and pentobarbitone, are increased by dihydropyridine calcium channel antagonists, such as nitrendipine (Dolin & Little, 1986). The present results show that nitrendipine greatly potentiated the anaesthetic properties of midazolam, while the calcium channel agonist, BAY K 8644, antagonised this effect.

Male TO mice, 30 - 35g, were used throughout, body temperatures being maintained at 37°C ± 0.5. All drugs were given by the intraperitoneal route, suspended in Tween 80, 0.5%. Nitrendipine was given 2h before, and BAY K 8644 immediately before, the benzodiazepines. General anaesthesia was measured by loss of righting reflex, at 5 min intervals until waking; groups of 10 mice were used for each dose. Dose response curves were generated by probit analysis, using at least four doses for each curve, and ED_{50} values calculated. For clarity only the values for 15 min after injection of midazolam are presented; other times showed the same pattern of parallel shifts in dose response curves.

ED_{50} values for midazolam, when combined with the following compounds:

	mg/kg	s.e.m.		mg/kg	s.e.m.
Tween vehicle	142.6	6.0	Tween vehicle	143.5	7.5
Nitrendipine 50 mg/kg	125.6**	5.5	BAY K 8644, 5 mg/kg	175.2**	7.2
Nitrendipine 100 mg/kg	15.9**	4.8	BAY K 8644, 10 mg/kg	195.1**	8.4

(** $P < 0.001$, Chi squared analysis)

Changes in central concentrations of the benzodiazepine did not account for the results; the brain concentrations (µg/g), 15 min after 200 mg/kg midazolam, were 0.67 ± 0.07 (8) with Tween vehicle; 0.74 ± 0.15 (8) with nitrendipine 100 mg/kg (2h pretreatment); and 0.50 ± 0.07 (7) with BAY K 8644 10 mg/kg. There were no significant differences from the vehicle group (Mann-Whitney 'U' test). In contrast, clonazepam did not show any anaesthetic actions, at doses of 100 or 400 mg/kg or 1 g/kg, or at the former two doses when combined with nitrendipine, 100 mg/kg. Clonazepam does not bind to the so-called peripheral benzodiazepine receptor (Marangos et al, 1982), which has been suggested to be associated with calcium channels (Mestre et al, 1985). Although the potencies of these two compounds in their other actions are not identical, such a large potentiating effect of nitrendipine as found with midazolam would have been expected to have been seen in the clonazepam studies, if it had been present. Although considerably more information is needed before definite conclusions can be reached, we suggest a tentative explanation for these results might be that the general anaesthetic actions of benzodiazepines involve neuronal calcium channels.

Little, H. J. & Bichard, A. J. (1984) Br. J. Anaesth., 56, 1153 - 1160
Dolin, S. J. & Little, H. J. (1986) Br. J. Pharmacol., 88, 909 - 914
Marangos, P. J. et al (1982) Mol. Pharmacol., 22, 26 - 32
Mestre, M. et al (1985) Life Sci., 36, 391 - 400

We thank the Wellcome Trust and the Brewers' Society for financial support.

8P

INTRATHECAL PROCTOLIN ALTERS TRH ANALOGUE (RX 77368) INDUCED BEHAVIOUR AND SPINAL CORD 5-HT AND TRH LEVELS IN THE RAT

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

Convincing evidence suggests that the peptide proctolin (Arg-Tyr-Leu-Pro-Thr-OH) is involved in invertebrate motor control (O'Shea, 1982), but recent findings also imply that this peptide may regulate mammalian spinal motor funtion. Proctolin-like immunoreactivity co-exists with 5-hydroxytryptamine (5HT) and thyrotrophin-releasing hormone (TRH) in rat bulbospinal neurones which terminate in the ventral horn (Holets et al., 1987) and iontophoretically applied proctolin depolarizes rodent motoneurones (White, 1985). The present study investigated both the behavioural effects of intrathecal (i.t.) proctolin, given alone and combined with a TRH analogue (RX 77368, pGln-His-3,3'-dimethyl-Pro-NH_2) and the effect of repeated proctolin administration on spinal cord levels of co-existent 5HT and TRH to further establish the possible motor role of this peptide.

An i.t. cannula was implanted in male Wistar rats under sodium methohexitone anaesthesia (60mgkg^{-1} i.p.) as previously described (Fone et al., 1987). Following 7 days recovery, the behavioural response to proctolin (10 and 100µg i.t.) was compared with that of saline for 30min post-injection (n=8). In separate rats (n=7) the number of wet-dog shakes (WDS) and the time spent forepaw-licking (FPL) induced in 30min following RX 77368 (0.05µg i.t.) were recorded; 30min after saline i.t. (day 7) and at the same time as (day 13) and 30min after proctolin (10µg i.t., day 19). Two further groups of rats (n=8 each) received repeated injections of proctolin (10µg i.t., twice daily) or saline for 5 days. At the end of this study the dorsal and ventral thoraco-lumbar spinal cord and brainstem levels of 5HT and 5-hydroxyindoleacetic acid (5HIAA) were determined using HPLC with electrochemical detection and TRH levels were measured by radioimmunoassay. Values given are mean±s.e.mean and Students' t-test was used for statistical analysis.

Proctolin alone failed to produce any marked change in behaviour compared with the effect of saline. However, the WDS (51±9 in 30min) and FPL (129±32s in 30min) behaviours induced by RX 77368 were attenuated following pretreatment with proctolin (18±10; $P<0.05$ and 23±8sec; $P<0.02$ respectively), while neither WDS (57±10) nor FPL (87±13s) were altered when proctolin was given at the same time as RX 77368. Repeated i.t. proctolin administration significantly reduced 5HT (27%; $P<0.01$), 5HIAA (18%; $P<0.05$) and TRH (26%; $P<0.05$) levels in the thoraco-lumbar ventral horn when compared with saline-treated control values, without affecting levels in the dorsal horn or the brainstem.

The finding that proctolin only attenuated the RX 77368-induced behaviours when it was administered 30min before the TRH analogue suggests that proctolin does not interact directly at the site at which the TRH analogue induces the observed behaviours. The selective reduction of ventral, but not dorsal, horn 5HT and TRH levels following chronic proctolin may result from their reduced synthesis and/or increased release and could be a functional consequence of the co-existence of proctolin with 5HT and TRH in bulbospinal raphe neurones.

We thank the M.R.C., the Wellcome Trust, Reckitt and Colman and Action Research for the Crippled Child for financial support.

Fone K.C.F., Bennett G.W. & Marsden C.A. (1987) Neuropharmacol. 26,1147-1155.
O'Shea M. (1982) Trends Neurosci. 5,69-73.
Holets V.R., Hokfelt T., Ude J., Eckert M., Penzlin H., Verhofstad A.A.J. & Visser T.J. (1987) Brain Res. 408,141-153.
White, S.R. (1985) Soc.Neurosci.Abstr. 11,91.

CHRONIC TRH ANALOGUE (CG 3509) INJECTION ALTERS THE BEHAVIOURAL RESPONSE TO INTRA-ACCUMBENS HISTAMINE ADMINISTRATION IN THE RAT

L.J. Bristow* & G.W. Bennett, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH, U.K.

We have reported previously that microinjections of histamine into rat nucleus accumbens induces a marked, H1-receptor mediated hyperactivity response (Bristow & Bennett, 1987). Furthermore, this arousal response is similar to that seen following intra-accumbens administration of TRH or its more stable analogues (Sharp et al, 1984). A number of in vitro reports suggest that histamine may interact with TRH in this region, for example (i) histamine releases TRH from synaptosomal preparations (Barraclough et al, 1983) and (ii) manipulation of endogenous brain histamine levels alters accumbens levels of TRH (Bennett et al, 1983). To further study possible histamine/TRH interaction in vivo, we have investigated the effect of repeated TRH analogue (CG 3509) injection on histamine induced behaviour.

Male Wistar rats (300 g) were anaesthetized with pentobarbitone (60 mg/kg, i.p.) and bilaterally implanted with guide cannulae for intra-accumbens injection. Drug-induced changes in activity were tested after an initial 7 day recovery period and were monitored using an Actimat activity meter for 60 mins (Sharp et al, 1984). All rats were initially tested for their responses to a) 0.9% saline (2 x 1 $\mu\ell$, day 1), b) histamine (10 μg, 2 x 1 $\mu\ell$, day 3), and c) CG 3509 (0.5 $\mu g/\mu\ell$, 2 x 0.5 $\mu\ell$, day 6). Rats were then chronically treated with either 0.9 % saline (2 x 0.5 $\mu\ell$) or CG 3509 (5 $\mu g/\mu\ell$, 2 x 0.5 $\mu\ell$), 5 bilateral microinjections given over 3 days (day 8 - 10). The behavioural responses to CG 3509 and histamine were then retested (Days 11, 12 respectively, i.e. 24 hrs and 48 hrs after last treatment).

Behavioural responses to both CG 3509 and histamine tested prior to treatment were not significantly different for the two groups of chronically treated rats. Chronic treatment with CG 3509 significantly enhanced the hyperactivity response induced by histamine compared to that seen before treatment (93%, $P<0.02$). Furthermore, chronic CG 3509 treatment was also associated with a significant reduction in CG 3509 induced hyperactivity (20%, $n = 7$, $P<0.05$). In contrast, chronic saline treatment did not affect either CG 3509 or histamine induced behaviours ($n = 8$).

These studies indicate that chronic treatment with the TRH analogue CG 3509 results in a behavioural tolerance to the peptide and that this is associated with a marked enhancement of histamine induced hyperactivity. The present results provide further evidence for interactions between the neuropeptide, TRH and histamine in mechanisms of arousal associated with the nucleus accumbens.

L.J. Bristow is a CASE student with SKF.

Barraclough, A. et al (1983) in 'Thyrotrophin Releasing Hormone': Griffiths, E.C. & Bennett, G.W. (eds.), pp 351-352. Raven Press, New York.
Bennett, G.W. et al (1983) Br. J. Pharmac. 80, 641P.
Bristow, L.J. & Bennett, G.W. (1987) Br. J. Pharmac. 91, 343P.
Sharp, T. et al (1984) Regulatory Peptides 9, 305-315.

10P

LACK OF TOLERANCE TO THE ANORECTIC EFFECTS OF OPIOID ANTAGONISTS IN THE RAT

G. Hewson, W.D. Turner, G.E. Leighton, R.G. Hill & J. Hughes
Parke-Davis Research Unit, Addenbrookes Hospital Site, Hills Road, Cambridge, CB2 2QB.

Tolerance to the anorectic actions of anti-obesity agents may limit their clinical usefulness and rapid tolerance has been reported to develop to the anorectic actions of dl-fenfluramine in rats (Rowland and Carlton, 1986). Opioid antagonists, such as naloxone, reduce food intake in both rats (Sanger et al, 1983) and man (Cohen et al, 1985) and may be of use in the treatment of obesity. To determine whether tolerance develops to the anorectic effects of these drugs, this study investigated the effects of subchronic administration of opioid antagonists on the intake of a palatable diet in non-deprived rats over a 5 day period.

Fifty Hooded Lister rats (Olac, starting weight 175-200 g), allowed free access to pellet food and water, were trained to eat a palatable diet (50 ml Nestles sweetened condensed milk; 150 ml tap water; 200 g powdered food blended together to form a mash) using a method similar to that described by Cooper et al (1985). Naloxone, naltrexone, WIN44441-3 (active isomer), WIN44441-2 (inactive isomer) or vehicle (distilled H_2O) were administered i.p. at a dose of 10 mg kg^{-1} (shown in acute dose-response studies to produce a substantial reduction in food intake) to groups of 10 rats once per day (between 1000-1130 hrs) for 5 days. Rats were presented with the palatable diet 10 min after drug administration and 30 min intake of the diet measured. Statistical comparisons between groups were made using Kruskal-Wallis one-way ANOVA followed by the Mann-Whitney U-test (two-tailed).

Naloxone, naltrexone, WIN44441-3 and WIN44441-2 all reduced the intake of the palatable diet (Table 1). Table 1 also shows that no tolerance developed to the anorectic effects of these drugs over the 5 days of the experiment.

Table 1 Lack of tolerance to the anorectic effects of opioid antagonists

	Mean food intake (g) (±SEM) on treatment day				
	1	2	3	4	5
vehicle	12.6±1.0	13.7±0.8	14.7±0.8	15.1±0.8	16.2±0.9
naloxone	5.7±1.0	5.5±0.9	4.1±0.7	3.1±0.8	3.7±0.8
naltrexone	6.4±0.5	5.5±0.4	4.4±0.7	4.5±0.8	5.8±1.0
WIN44441-3	2.5±0.5	3.2±0.8	2.5±0.4	3.2±0.9	3.9±1.3
WIN44441-2	3.4±0.6	5.3±1.5	4.6±1.1	4.1±1.2	4.5±1.0

All drug treated groups significantly different from the vehicle treated groups $p<0.01$ Mann-Whitney U-test.

Body weight gain from day 1 to day 5 was also significantly reduced by all 4 drugs (vehicle +21±1 g; naloxone +11±3 g **; naltrexone +14±2 g **; WIN44441-3 +5±3 g **; WIN44441-2 +8±4 g **; ** $p<0.01$ different from vehicle group).

It is concluded that tolerance does not develop to the anorectic effects of opioid antagonists after once daily administration over a period of 5 days. The finding that both the active and inactive isomers of WIN44441 reduced food intake to a similar degree, suggests that not all of the anorectic effects of this compound are mediated at opioid receptors.

Cohen, M.R. et al (1985) Psychosom. Med., 47, 132-138.
Cooper, S.J. et al (1985) Neuropharmacology, 24, 877-883.
Rowland, N.E. & Carlton, J. (1986) Appetite, 7 Suppl., 71-83.
Sanger, D.J. et al (1983) Drug Devel. Res., 3, 137-142.

MECHANISMS OF ANORECTIC ACTIVITY OF SEROTONIN UPTAKE INHIBITORS

I. Angel*, Marie-Anne Taranger, B. Scatton and S.Z. Langer, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France

Various biochemical and pharmacological properties of several serotonin uptake inhibitors were compared with their anorectic potencies and in reference to the novel compound SL 81.0385 (4-[(2-naphthalenyl)methoxy]piperidine).

SL 81.0385, a potent serotonin uptake inhibitor in vitro (inhibition of serotonin uptake into rat hypothalamic synaptosomes, IC_{50} = 18 nM) and in vivo (antagonism of p-chloroamphetamine induced depletion of cerebral serotonin levels, ED_{50} = 2.5 mg/kg i.p.), demonstrates anorectic activity (ED_{50} = 4 mg/kg i.p.) which was potentiated by 5-hydroxytryptophan and blocked by metergoline. We compared the anorectic activities of fluoxetine, fluvoxamine, zimelidine, indalpine, chlorimipramine and SL 81.0385 with their interactions on transporter sites. A good correlation ($r = 0.98$, $p < 0.01$) was obtained between the ED_{50} values of anorectic action and the ED_{50} values of serotonin uptake inhibition in vivo of these drugs, indicating that their anorectic action may be directly mediated through the inhibition of serotonin uptake.

The interaction of these drugs with an anorectic recognition site was further studied. This site which is labelled with [^{3}H]mazindol in rat hypothalamic membranes, is a low-affinity (K_d = 10 µM), high capacity and Na^+-independent site, primarily located in the medial hypothalamus and in the brain-stem, is believed to mediate the anorectic action of non-serotonergic anorectic compounds (Angel et al., 1987a).

The majority of the uptake inhibitors tested displaced [^{3}H]mazindol from its binding to the anorectic recognition site. Excluding the prodrug zimelidine (IC_{50} > 100 µM) a good correlation ($r = 0.77$, $p < 0.05$) was obtained between the affinities of these drugs to the [^{3}H]mazindol binding and their anorectic potencies indicating that the anorectic activity of these drugs may also be mediated through this site.

The regulation of the Na^+-independent [^{3}H]mazindol binding site by glucose (Angel et al., 1985; 1986) and by serotonin (Angel et al., 1987b) and the indication that it may mediate the anorectic activity of serotonergic drugs may reflect common sites of interaction for the regulation of carbohydrate intake.

Angel, I., Hauger, R.L., Luu, M.D., Giblin, B., Skolnick, P. & Paul, S.M. (1985) Proc. Natl. Acad. Sci. USA, 82, 6320-6324.
Angel, I., Kiss, A., Stivers, J.A., Skirboll, L., Crawley, J.N. & Paul, S.M. (1986) Brain Res. Bull., 17(6), 873-877.
Angel, I., Luu, M.D. & Paul, S.M. (1987a) J. Neurochem. 48(2), 491-497.
Angel, I., Janowsky, A. & Paul, S.M. (1987b) Brain Res., in press.

12P

[^{3}H]-PAROXETINE BINDING TO PLATELETS FROM HEALTHY VOLUNTEERS AND DEPRESSED PATIENTS

Anne-Marie Galzin[1]*, Marie-France Poirier[2], H. Loo[2], D. Sechter[3], E. Zarifian[4] & S.Z. Langer[1], [1]Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France; [2]Hôpital Sainte-Anne, 1, rue Cabanis, 75014 Paris, France; [3]Centre de Soins Ulysse Trélat, B.P. 26, 59872 Saint-André, France; [4]Centre Esquirol, C.H.U. Côte de Nacre, 14033 Caen Cédex, France.

Radioligand ([^{3}H]-imipramine) binding to the 5-HT transporter in platelets has been proposed as a biological marker in depression. In most studies, the B_{max} of [^{3}H]-imipramine binding on platelet membranes from untreated severely depressed patients was found to be significantly decreased when compared with healthy volunteers (for review, see Langer et al., 1987). However, these studies have been performed exclusively using [^{3}H]-imipramine and at an incubation temperature of 0°C. Radioligand binding studies to the 5-HT transporter at 20°C or 37°C have now become possible, using a new selective and non-tricyclic 5-HT uptake inhibitor, [^{3}H]-paroxetine. It has been shown that the [^{3}H]-paroxetine and [^{3}H]-imipramine binding sites within the human platelet 5-HT transporter complex are at least closely related, if not identical (Schoemaker and Langer, 1987 for review). Therefore, it was considered of interest to study [^{3}H]-paroxetine binding to platelets of depressed patients and healthy volunteers.

We studied a group of untreated depressed patients (6 male and 10 female, 19 to 77 years old; mean age: 45.5±4) who were suffering from a major depressive episode according to the DSM III classification (DSM III 296-[22, 23, 32, 33, 34]). The mean Hamilton rating score was: 29.8±2.0; NIMH 1967. Patients were chosen to fulfil the criteria of washout described previously (Poirier et al., 1984). A control group free of somatic or psychiatric disorders, and receiving no psychoactive medication was matched as closely as possible for age, sex and sampling time with the depressed population. Blood samples were collected at 8.00 a.m. and [^{3}H]-paroxetine binding assays were performed according to Segonzac et al. (1987) at 20°C with 10 µM fluoxetine to define non-specific binding. Under these conditions, there was no difference in [^{3}H]-paroxetine binding at 20°C between control and depressed patients groups (B_{max}= 895±52 fmol/mg prot (n=13) in control group; B_{max}= 874±44 fmol/mg prot (n=16) in depressed patients; K_d= 0.12±0.01 nM in control group; K_d= 0.14±0.02 nM in depressed patients).

These results are in contrast with the 47% decrease in the B_{max} of [^{3}H]-imipramine binding to platelets from depressed patients (Poirier et al., 1986). The apparent discrepancy between our present results obtained using [^{3}H]-paroxetine binding at 20°C and our previous data obtained using [^{3}H]-imipramine at 0°C may be explained by differences between the two recognition sites associated with the 5-HT transporter (Mellerup et al., 1984). Alternatively, it could be related to the difference in the incubation temperature. The present findings need to be confirmed in studies in which [^{3}H]-imipramine and [^{3}H]-paroxetine binding in parallel are determined in the same subjects population.

Langer, S.Z. et al. (1987) J. Rec. Res. 7, 499.
Mellerup, E.T. et al. (1984) Eur. J. Pharmacol. 106, 411.
Poirier, M.F. et al. (1984) Eur. J. Pharmacol. 106, 629.
Poirier, M.F. et al. (1986) Psychopharmacol. 89, 456.
Schoemaker, H. and Langer, S.Z. (1987) Neuromethods vol. 6, in press.
Segonzac, A. et al. (1987) J. Neurochem., 48, 331.

A COMPARISON OF ELECTRICAL- AND POTASSIUM-EVOKED TRITIUM AND TRITIATED SEROTONIN RELEASE FROM RAT HYPOTHALAMUS SLICES

A.M.BROADHURST*, G.W.BROWN, C.MORET and M.BRILEY, Centre de Recherche Pierre Fabre, 17 Avenue Jean Moulin, 81106 Castres, France.

The assay of evoked tritium release from prelabelled brain preparations has been a major factor in the development of central auto- and heteroreceptor theory (Chesselet, 1984). The interpretation of results obtained with tritium release, however, is often based on the assumption that the majority of the evoked tritium corresponds to unmetabolised transmitter. In the present communication we present the results of experiments designed to measure and compare potassium(K^+)- (25mM,3min) and electrically-evoked (3Hz, 20 mA, 2 msec, 2 min) release of both total tritium and ^{3}H-5HT from superfused, prelabelled rat hypothalamic slices. Both stimulation parameters were chosen so as to evoke 1.5-2.5% of total tissue tritium at the first stimulation (S1).

All details of slice preparation, superfusion and electrical stimulation parameters have been previously described (Langer and Moret,1982). Each aliquot of superfusate was divided into two ; one was assayed for total tritium, the other for ^{3}H-5HT after extraction on disposable strong cation exchange columns as previously used for the assay of endogenous noradrenaline release (Broadhurst, 1986). Results were expressed either as percent fractional release of total tritium and subsequently as a ratio of the first and second stimulations (S2/S1) or as the percentage of ^{3}H-5HT in each aliquot.

The evoked release of tritium was comparable whatever the type of stimulation used (Table 1). Differences were seen, however, when ^{3}H-5HT was measured in the same fractions. These differences in the fractional release and the S2/S1 ratio of electrically- and K^+-evoked release of ^{3}H-5HT were fully accounted for by the percentage of ^{3}H-5HT found in each fraction (Table 1).

Table 1

		-----^{3}H-----		--^{3}H-5HT---		----%5HT----	
Stimulation	n	S1	S2/S1	S1	S2/S1	S1	S2
Electrical	16	1.78±0.18	1.16±0.06	0.60±0.04	1.06±0.10	37.8±4.2	35.5±6.7
Potassium	6	2.49±0.32	1.17±0.12	2.40±0.26	0.81±0.07	103±16	72.5±12

Thus, even under conditions found to evoke the release of a similar proportion of tissue tritium, the percentage of ^{3}H-5HT released was far greater when K^+-evoked stimulation was used. These findings should be borne in mind when comparing data from experiments using these different methods of stimulation.

Broadhurst A.M.(1986) Br.J.Pharmac 88 376P
Chesselet M.F.(1984) Neuroscience 12(2) 347-375
Langer S.Z.,Moret C.(1982) J.Pharm.Exp.Ther 222 220-226

14P

MODULATION OF THE ELECTRICALLY-EVOKED RELEASE OF [^{3}H]-5-HT FROM SLICES OF HUMAN FRONTAL CORTEX

Anne-Marie Galzin[1]*, J.P. Chodkiewicz[2], Marie-France Poirier[3], H. Loo[3], F.X. Roux[2], A. Redondo[2], A. Lista[1], Régine Ramdine[1], P. Blier[1] and S.Z. Langer[1], [1]Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France; [2]Service de Neurochirurgie, Hôpital Sainte-Anne, 1, rue Cabanis, 75014 Paris, France; [3]Service de Psychiatrie, Hôpital Sainte-Anne, 1, rue Cabanis, 75014 Paris, France.

It is well established that presynaptic inhibitory 5-HT autoreceptors present on 5-HT nerve endings can modulate the release of 5-HT in various brain regions of mammalian species. In the rat, terminal 5-HT autoreceptors belong to the 5-HT$_{1B}$-subtype. Although radioligand binding studies have not revealed the presence of 5-HT$_{1B}$ binding sites in human brain (Hoyer et al., 1986), the existence of presynaptic terminal 5-HT autoreceptors of an uncharacterized pharmacological type has been reported (Schlicker et al., 1985). Moreover, in the rabbit (Limberger et al., 1986) or guinea-pig brains (Middlemiss and Bremer, 1987) the terminal 5-HT autoreceptor is distinct from 5-HT$_{1A}$, $_{1B}$, $_{1C}$, 5-HT$_2$ or 5-HT$_3$ receptors. We thus decided to study the effects of selective 5-HT agonists and antagonists on the electrically-evoked release of [^{3}H]-5-HT from human frontal cortex slices.

Specimens of frontal cortex were obtained from 3 male and 1 female patients (47-64 years old) undergoing neurosurgery. Immediately after removal, the tissue was placed in Krebs' solution at 4°C, and transferred to the laboratory. Brain slices (0.4 mm thickness) were prepared and incubated for 30 min in Krebs' solution (37°C) containing 0.1 µM [^{3}H]-5-HT. The delay between removal and beginning of the labelling with [^{3}H]-5-HT was 25 min. The slices were superfused at 0.5 ml/min with Krebs' solution. Two periods of electrical stimulation (3 Hz, 2 msec, 30 mA, for 2 min) were applied 90 min (S_1) and 134 min (S_2) after the onset of superfusion. Drugs were added 20 min before S_1 or S_2.
Under control conditions, the fractional release of radioactivity evoked by electrical stimulation was 0.80±0.09% of radioactivity present in the tissue, with a S_2/S_1 ratio of 1.38±0.12 (n=15). Selective 5-HT uptake inhibitors like paroxetine or SL 81.0385 increased significantly the electrically-evoked release of [^{3}H]-5-HT. When added 20 min before S_2, the non-selective 5-HT agonist 5-carboxamidotryptamine (5-CT) inhibited the electrically-evoked release of [^{3}H]-5-HT (S_2/S_1 = 0.35±0.10, n=3 and S_2/S_1 = 0.12±0.09, n=4 for 5-CT 0.01 and 0.03 µM, respectively). The potent inhibition of the release of [^{3}H]-5-HT observed in the presence of 0.03 µM 5-CT was completely antagonized when the non-selective 5-HT receptor antagonist methiothepin (1 µM) was added to the medium 20 min before S_1 (S_2/S_1 = 1.37±0.22, n=4 $p<0.01$). The selective 5-HT$_{1A}$ agonist 8-OH-DPAT (0.1 µM) did not modify the electrically-evoked release of [^{3}H]-5-HT in human brain slices (S_2/S_1 = 1.03±0.21, n=5). The selective 5-HT$_{1B}$ agonist RU 24969 inhibited in a concentration-dependent manner the [^{3}H]-5-HT overflow (S_2/S_1 = 0.77±0.15, n=4, and S_2/S_1 = 0.48±0.14, n=4 in the presence of 0.01 and 0.1 µM RU 24969 respectively, which represents 44 and 65% inhibitions). This implies that the IC_{50} for this drug should be between 0.01 and 0.1 µM. In contrast, the IC_{50} of RU 24969 on the electrically-evoked release of [^{3}H]-5-HT in rat hypothalamic slices was 0.0036 µM±0.0003.

These results indicate that the release of 5-HT from human frontal cortex is modulated by an inhibitory 5-HT receptor which is different from the 5-HT$_{1A}$ subtype. The difference of potency of RU 24969 between rat hypothalamic slices (terminal 5-HT$_{1B}$ autoreceptor) and human frontal cortex slices support the view that the human 5-HT receptor modulating the release of 5-HT may be different from the 5-HT$_{1B}$ subtype.

Hoyer, D. et al. (1986) Brain Res. 376, 85.
Limberger, N. et al. (1986) Naunyn-Schmiedeberg's Arch. Pharmacol., 332, 324.
Middlemiss, D. and Bremer, M.E. (1987) Br. J. Pharmacol., in press.
Schlicker, E. et al. (1985) Brain Res. 331, 337.

THE RELAXANT AND THE SPASMOGENIC EFFECTS OF SOME XANTHINE DERIVATIVES IN GUINEA-PIG ISOLATED TRACHEALIS

J.P. Boyle*, J. Cortijo, J.M. Davies, R.W. Foster, [1]P. Hofer & R.C. Small, Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester Medical School, Oxford Road, Manchester M13 9PT, U.K. and [1]Mundipharma AG, Ruemelinbachweg 12, CH-4054, Basel, Switzerland.

In addition to their long-recognised ability to relax airways smooth muscle in vitro, methylxanthines such as caffeine can also evoke spasm by liberating Ca^{2+} from intracellular sites of sequestration (Ito & Itoh, 1984). Using isometric recording of tension changes from guinea-pig trachealis (Foster et al., 1983) we have sought to establish optimal conditions for observing the spasmogenic effects of xanthine derivatives and to compare their relaxant and spasmogenic actions.

Preliminary experiments at 37°C revealed that caffeine (10 mM) abolished spontaneous tracheal tone. Tissue treatment with 2.8 μM indomethacin abolished tone and thereby abolished relaxant responses to caffeine. Spasmogenic responses to caffeine appeared and increased in amplitude as the indomethacin-treated tissues were cooled to 12°C. Spasmogenic actions of xanthine derivatives were subsequently studied in indomethacin-treated tissues maintained at 12°C whilst relaxant actions were studied in indomethacin-free tissues at 37°C.

All xanthines tested had log concentration/relaxation curves of similar shape and maximal effect. The relative order of relaxant potency differed markedly from that observed for the spasmogenic effects of the xanthines (Table 1). Forskolin (100 pM- 1 μM) caused concentration/dependent relaxation to the same maximum as the xanthines but was devoid of spasmogenic activity in concentration up to 100 μM.

Table 1. pD_2 values for xanthine derivatives acting as relaxants or spasmogens in trachealis. Data represent mean ± s.e.mean. Figures in parenthesis = number of observations.

	Suppression of tone at 37°C	Spasm in presence of 2.8 μM indomethacin at 12°C
Caffeine	3.91 ± 0.03 (6)	3.30 ± 0.05 (6)
Enprofylline	5.59 ± 0.02 (6)	3.20 ± 0.05 (12)
Theobromine	3.59 ± 0.05 (6)	3.51 ± 0.03 (15)
Theophylline	4.36 ± 0.03 (8)	3.42 ± 0.06 (6)
Xanthine	2.64 ± 0.05 (6)	2.33 ± 0.03 (6)
TMX	1.75 ± 0.03 (6)	< 2.0 (6)

These findings are consistent with the hypothesis that the relaxant and spasmogenic actions of the xanthines have different underlying mechanisms. The ability of 1,3,7,9-tetramethylxanthinium (TMX) to evoke spasm at concentrations similar to those causing relaxation suggests that this substance gains access to the cell cytoplasm. If so, it weakens the argument (Persson, 1985) that the relaxant action of TMX and other methylxanthines is mediated by a receptor located on the cell surface.

Supported by the Asthma Research Council, the British Lung Foundation, Napp Laboratories Ltd and the North Western Regional Health Authority.

Foster, R.W. et al (1983) Br. J. Pharmac., 79, 255-263
Ito, Y. & Itoh, T. (1984) Br. J. Pharmac., 83, 667-676
Persson, C.G.A. (1985) In Anti-Asthma Xanthines and Adenosine. ed. Andersson, K.E. & Persson, C.G.A. pp 61-83. Amsterdam: Excerpta Medica.

16P

NEUROGENIC ELECTRICAL SLOW WAVES RECORDED FROM CIRCULAR SMOOTH MUSCLE CELLS OF MOUSE COLON

R.A.R. Bywater, [1]R.C.Small* & G.S. Taylor, Neuropharmacology Group, Department of Physiology, Monash University, Clayton, Victoria 3168, Australia and [1]Smooth Muscle Research Group, Department of Physiological Sciences, University of Manchester Medical School, Oxford Road, Manchester M13 9PT, U.K.

Mouse isolated colon was opened by cutting longitudinally alongside the point of mesenteric attachment. Half-width strips of the tissue were then set up for electrophysiological recording in Krebs solution containing 1.0 μM nifedipine to minimise tissue movement. Microelectrode recordings of membrane potential were made from cells of the circular smooth muscle layer approximately 2.5 cm from the anus. Transmural electrodes located 1 mm oral to the point of recording were used to deliver single stimuli (0.6 ms duration; frequency 0.25 Hz) to the intramural neurones.

As reported by Okasara et al (1986) the resting membrane potential of the circular smooth muscle cells was of the order -50 mV. However, in the presence or absence of transmural stimulation, many cells exhibited slow waves of depolarisation (amplitude 5-22 mV; duration 30-150s) which were surmounted by a series of rapid oscillations of membrane potential. Inhibitory junctional potentials (IJPs) evoked by transmural stimulation were reduced in amplitude during the depolarising phase and the plateau phase of the slow wave.

Tetrodotoxin (3.1 μM) abolished the slow waves, their attendant membrane potential oscillations and the excitatory junctional potentials (EJPs) and IJPs evoked by transmural stimulation. Hexamethonium (500 μM) abolished the slow waves and the oscillations, but did not abolish evoked IJPs. In the presence of hexamethonium IJP amplitude became relatively constant. Morphine (1 and 10 μM) abolished the slow waves and the oscillations without abolishing the evoked IJP. The suppressant effects of morphine (1 μM) on the slow waves and oscillations could be offset by naloxone (5 μM). Atropine (3.5 μM) abolished the membrane potential oscillations but did not abolish the underlying slow wave or its effect on IJP amplitude. Addition of morphine (10 μM) suppressed the atropine-resistant component of the slow wave and IJP amplitude then became relatively constant. Electrotonic potentials recorded from the circular smooth muscle cells were reduced in amplitude during the depolarising phase and early plateau phase of the slow wave.

These findings suggest that neural activity is responsible for the slow waves and membrane potential oscillations observed in the circular smooth muscle of mouse colon. The neural mechanisms involve transmission across a cholinergic synapse with postsynaptic nicotinic cholinoceptors. The oscillations of membrane potential may result from the activity of postganglionic cholinergic neurones but the underlying slow wave may have both cholinergic and non-cholinergic components. An increase in membrane conductance occurring during the slow wave may partly account for the observed attenuation of evoked IJPs.

RCS was supported by a travel grant from the Wellcome Ramaciotti Foundations.

Okasora, T., Bywater, R.A.R. & Taylor, G.S. (1986) Gastroenterology, 90, 1964-1971

THE ACTION OF GLYCERYL TRINITRATE AND SODIUM NITROPRUSSIDE ON RAT AORTA: A COMPARISON WITH NICORANDIL AND BRL34915

D.T. Newgreen*, K.M. Bray, J.S. Southerton & A. H. Weston, Smooth Muscle Research Group, Physiological Sciences Department, Manchester University Medical School, Oxford Road, Manchester M13 9PT

Glyceryl trinitrate (GTN) and sodium nitroprusside (SNP) are vasodilator drugs thought to act via guanylate cyclase stimulation (Katsuki et al., 1977; Kukovetz et al., 1979). BRL34915 and nicorandil open K-channels and hyperpolarize the membrane of vascular and other smooth muscles (Hamilton et al., 1986; Weir & Weston, 1986). In addition, nicorandil increases intracellular cyclic guanosine monophosphate (cGMP) concentrations in vascular smooth muscle (Holzmann, 1983). In the present study, the effects of methylene blue and M&B 22948 on the relaxant effects of GTN, SNP, BRL34915 and nicorandil were examined. Furthermore, the effects of the four relaxants on changes in cyclic adenosine monophosphate (cAMP) and cGMP concentrations were investigated. These studies were designed to clarify the mechanism of action of GTN and SNP and to study possible links between cyclic nucleotide changes and the opening of K-channels in this tissue.

Four endothelium-free strips were prepared from the thoracic aorta of male Sprague-Dawley rats (300-500g). For tissue bath studies, tissues were mounted for isometric recording in MOPS-buffered physiological salt solution (MOPS PSS) at 37°C. For cyclic nucleotide measurements using radioimmunoassay, the strips were impaled on syringe needles and immersed in MOPS PSS at 37°C.

GTN, SNP, BRL34915 and nicorandil produced complete relaxation of 20 mM KCl contractions (IC_{50} values, 6.4 nM, 8.1 nM, 79.4 nM and 5.0 μM respectively). Methylene blue (10 μM) inhibited relaxant responses to GTN, SNP and nicorandil producing rightwards shifts in the concentration/response curves to these agents (144.6, 10.7 and 3.5 fold, respectively) but had no effect on responses to BRL34915. The phosphodiesterase inhibitor 2-O-propoxyphenyl-8-azapurin-6-one (M&B 22948) (100 μM) potentiated the relaxant effect of GTN, producing a 15 fold leftwards shift in the GTN concentration/response curve, but had no effect on relaxant responses to SNP, nicorandil or BRL34915. GTN and SNP produced increases in cGMP which correlated with the relaxant response but had no effect on cAMP concentrations. Nicorandil dose-dependently increased cGMP concentrations but had no effect on cAMP levels while BRL34915 had no effect on either cAMP or cGMP concentrations.

It is concluded that GTN, SNP and nicorandil stimulate guanylate cyclase turnover and increase intracellular cGMP concentration in rat aorta. However, the results with methylene blue and M&B 22948 suggest that the action of GTN is more closely linked to changes in cGMP than that of nicorandil or SNP (see Weir & Weston, 1987, this meeting). The actions of GTN, SNP and nicorandil are not associated with any change in intracellular cAMP concentration. Changes in cAMP or cGMP concentration have no role in the relaxant action of BRL34915 on the rat aorta.

Further work is in progress to investigate the relationship between potassium channels and changes in cGMP concentration by studying the effects of the four relaxants used in this study on ^{86}Rb efflux from the rat aorta.

Supported by SERC case awards to D.T.N. and J.S.S. K.M.B. was supported by a Ciba-Geigy studentship.

Hamilton, T., Weir, S.W. & Weston, A.H. (1986) Br. J. Pharmac. 88, 103-111.
Holzmann, S. (1983) J. cardiovasc. Pharmac. 5, 364-370.
Katsuki, S., Arnold, W., Mittal, C. & Murad, F. (1977) J. Cyc. Nucl. Res. 3, 23-35.
Kukovetz, W.R., Holzmann, S., Wurm, A. & Poch, G. (1979) Naunyn-Schmiedeberg's Arch. Pharmac. 310, 129-138.
Weir, S.W. & Weston, A.H. (1986) Br. J. Pharmac. 88, 121-128.
Weir, S.W. & Weston, A.H. (1987) This meeting.

18P

EFFECT OF APAMIN ON THE INHBITORY ACTIONS OF SODIUM NITROPRUSSIDE AND SODIUM AZIDE ON THE GUINEA-PIG TAENIA CAECI

S.W. Weir* & A.H. Weston[1]. Cardiovascular Department, Preclinical Research, Sandoz Ltd., CH-4002 Basle, Switzerland.[1]Smooth Muscle Research Group, Department of Physiological Sciences, Medical School, University of Manchester, Manchester M13 9PT, U.K.

The exact mechanism by which sodium nitroprusside (NP) relaxes smooth muscle is still a matter of debate. It has been suggested that the NO group within its structure contributes in part to its relaxant effect by activation of guanylate cyclase to produce an increase in cGMP levels (Rapoport & Murad, 1983). However, in some vascular tissues, NP is capable of producing a hyperpolarisation of the cell membrane, although it is not clear whether this is due to an increase in K^+ permeability or a decrease in Cl^- permeability (Kreye, 1980). In the present study, we have investigated the effect of NP on K^+ permeability in the guinea-pig taenia caeci and a comparison has been made with sodium azide which is also known to activate guanylate cyclase (Rapoport & Murad, 1983).

Male guinea-pigs (250-350g) were killed, the taenia caeci was exposed, cut into equal segments and incubated in a Krebs-Henseleit solution (KHS) under isotonic conditions and a 1g load. After an initial equilibration period of 45 min, tissues were primed with noradrenaline (8×10^{-7}M) for 1 min to optimise the resting tone (Weir & Weston, 1986). A further equilibration period of 45 min was allowed before the tissues were exposed to either NP, sodium azide or noradrenaline using a cumulative concentration-effect protocol. At the end of the experiment, tissues were challenged with papaverine (10^{-4}M) to determine 100% relaxation. NP (10^{-8} - 10^{-4}M), sodium azide (10^{-8}-10^{-4}M) and noradrenaline ($1\text{-}32 \times 10^{-7}$M) produced a concentration-dependent relaxation of spontaneous tone which was rapid in onset. None of the relaxants was capable of producing a 100% relaxation. In the presence of apamin (10^{-7}M), the relaxations produced by noradrenaline were completely abolished, those to NP were inhibited with the maximum response reduced by 50%. The relaxations to sodium azide were totally unaffected by apamin.

After an inital equilibration period and subsequent exposure to noradrenaline (8×10^{-7}M) for 1 min, tissues were loaded with $^{86}Rb^+$(5μCi/ml) for 2h. The $^{86}Rb^+$ was then allowed to efflux from the tissues into KHS for 10 min before exposure to apamin (10^{-7}M) or vehicle for the next 20 min. At the end of this period, taeniae were challenged with either NP (10^{-5}M), sodium azide (10^{-5}M) or noradrenaline (32×10^{-7}M). Both NP and noradrenaline produced an increase in the $^{86}Rb^+$ efflux rate constant which was totally abolished by apamin. Sodium azide was without effect on the $^{86}Rb^+$ rate constant.

It is concluded that the relaxant effect of NP on the spontaneous tone of the taenia caeci is partly due to the opening of the apamin-sensitive K^+ channel. The inhibitory action of sodium azide is not associated with the opening of $^{86}Rb^+$ permeable K^+ channels.

Kreye, V.A.W. (1980) TIPS. 1: 384-388.
Rapoport, R.M. & Murad, F. (1983) J. Cyc. Nuc. Prot. Phosphoryl. Res. 9: 281-296.
Weir, S.W. & Weston, A.H. (1986) Br. J. Pharmac. 88: 113-120.

COMPARISON OF ^{86}Rb AND $^{42}K^+$ EFFLUXES STIMULATED BY THE K^+ CHANNEL OPENER BRL 34915 IN VASCULAR SMOOTH MUSCLE

U. Quast (Introduced by G. Engel), Preclinical Research, Sandoz Ltd, CH4002-Basle, Switzerland.

The novel vasorelaxant agent BRL 34915 has been proposed to act by increasing the K^+ permeability (P_K) of vascular smooth muscle (VSM) thus hyperpolarizing the tissue (Hamilton et al., 1986). Evidence for an increase in P_K was obtained by measuring membrane potential (Hamilton et al., 1986) or $^{86}Rb^+$ efflux (Hamilton et al. 1986 ; Quast, 1987); however, these effects were observed only at concentrations of BRL 34915 considerably higher than those required to elicit the vasorelaxing response. One possible reason for the inability to detect stimulation of $^{86}Rb^+$ efflux at low concentrations of BRL 34915 might be that the K^+ channels opened by the drug are highly selective for K^+ over Rb^+. In order to clarify this point we have compared the effect of BRL 34915 on the effluxes of $^{86}Rb^+$ and $^{42}K^+$ from VSM.

Strips from rat aorta or guinea-pig portal veins (gppv) were incubated in HEPES-buffered PSS (pH 7.2) supplemented with $^{42}K^+$ (5 µCi/ml) or $^{86}Rb^+$ (5 µCi/ml) or both (double labelling experiments). Efflux of radioactivity was measured in a superfusion capillary (Quast, 1987) at 37°C (rat aorta) or 32°C (gppv), the latter in the presence of 0.3 or 0.5 µM of the dihydropyridine Ca^{2+} antagonist PN 200-110 (Hof et al., 1984) in order to abolish myogenic activity . $^{42}K^+$ data were corrected for the short half life of the isotope; double labelling experiments were evaluated essentially as described by Smith et al. (1986). Flux data are expressed as the rate constants, k, of the respective isotope efflux; drug effects on k are given as Δ % of the predrug value at the peak of the effect.

The results obtained in separate $^{42}K^+$ and $^{86}Rb^+$ efflux experiments are summarized in the following table:

	rat aorta		gppv (+PN 200-110)	
BRL (µM)	Δk_K(%)	Δk_{Rb}(%)	Δk_K(%)	Δk_{Rb}(%)
0.03	3 ± 1	0 ± 0	7 ± 1	2 ± 1
0.1	20 ± 1	5 ± 1	16 ± 1	9 ± 1
1	137 ± 21	90 ± 8	87 ± 11	55 ± 5
10	324 ± 38	185 ± 15	234 ± 16	176 ± 18

In double labelling experiments in gppv it is shown that the K^+ channel inhibitors tetraethylammonium (TEA) and 3,4 diaminopyridine (DAP) inhibited the effluxes of $^{42}K^+$ and $^{86}Rb^+$ stimulated by 6 µM BRL 34915, to a similar degree.

It is concluded that (i) a small increase in $^{42}K^+$ efflux can be detected at the concentration of BRL 34915 (30 nM) where the spontaneous activity of gppv is inhibited by ≈ 80 %, (ii) the K^+ channels opened by BRL 34915 are more permeable for K^+ than for Rb^+ but that $^{86}Rb^+$ efflux appears to qualitatively reflect the BRL 34915-induced changes in P_K and (iii) the increases in permeability for $^{86}Rb^+$ and $^{42}K^+$ elicited by 6 µM BRL 34915 are not differentially affected by TEA or DAP.

Hamilton, T.C., Weir, S.W. & Weston, A.H. (1986) Br. J. Pharmac. 88, 103-111.
Hof, R.P., Scholtysik, G., Loutzenhiser, R., Vuorela, H.J. & Neumann, P. (1984) J. Cardiovasc. Pharmac. 6, 399-406.
Quast, U. (1987) Br. J. Pharmac. 91, 569-578.
Smith, J.M., Sanchez, A.A. & Jones, A.W. (1986) Blood Vessels 23, 297-303.

20P

HYPERGLYCAEMIA INDUCED BY α_2-ADRENOCEPTOR STIMULATION: PHARMACOLOGICAL CHARACTERIZATION

I. Angel*, Sylvie Bidet, Nicole Duval & S.Z. Langer, Department of Biology, Laboratoires d'Etudes et de Recherches Synthélabo (L.E.R.S.), 58, rue de la Glacière, 75013 Paris, France.

Alpha-adrenergic stimulation, which induces an hyperglycaemic response in several animal species including man, has been shown under in vivo conditions and in isolated pancreatic islets to inhibit insulin secretion through α_2-adrenoceptors located on islet ß-cells (Porte and Williams, 1966; Nakaki et al., 1981; Langer et al., 1983).

The administration of the selective α_2-adrenoceptor agonist UK 14.304 (5-bromo-6-(2-imidazolin-2-ylamino)-quinoxaline) induced in the mouse, rat and anaesthetized dog a dose-dependent hyperglycaemic response. Similar hyperglycaemic effects were observed with the selective α_2-adrenoceptor agonists clonidine and guanabenz whereas smaller effects were observed with (-)adrenaline and no significant effects with the α_1-receptor agonist methoxamine.

The hyperglycaemic response to UK 14.304 was present only in fed mice, and was absent in mice that were food-deprived for 24 h. This hyperglycaemic effect of UK 14.304 was potently antagonized (ED_{50} < 0.5 mg/kg i.p.) by the centrally and peripherally acting α_2-adrenoceptor antagonists rauwolscine, yohimbine, idazoxan and phentolamine, by the peripheral α_2-adrenoceptor antagonist benextramine (ED_{50} = 5.5 mg/kg i.p.) but not by prazosin (α_1-selective) or propranolol (ß-adrenergic) (at 3 mg/kg i.p.).

The hyperglycaemic response to UK 14.304 was also attenuated by the sulphonylurea derivatives glybenclamide (ED_{50} = 0.5 mg/kg i.p.) and tolbutamide (ED_{50} = 12.0 mg/kg i.p.) and by DG 5128 (ED_{50} = 12.0 mg/kg i.p.), a compound possessing α_2-antagonist properties.

The hyperglycaemic response to UK 14.304 was not affected by depletion of catecholamine stores either by pretreatment with reserpine, α-methylparatyrosine or DSP4. Furthermore, it was not attenuated by adrenalectomy nor by ganglionic blockade with chlorisondamine.

However, pretreatment with streptozotocin, both at prediabetic (100 mg/kg i.p.) or diabetic (200 mg/kg i.p.) doses, completely abolished the hyperglycaemia induced by UK 14.304.

It is suggested that this hyperglycaemic response is mediated primarily via stimulation of postsynaptic α_2-adrenoceptors in the pancreatic ß-cells which triggers an inhibition of insulin release.

Langer, J., Panton, V. & Zielmann, S. (1983) Br. J. Pharmacol. 79, 415-420.
Nakaki, T., Nakadate, T., Kunio, I. & Kato, R. (1981) J. Pharmacol. Exp. Ther. 216, 607-617.
Porte, D. & Williams, R.H. (1966) Science 152, 1248-1250.

AN ADRENOCEPTOR WITH DISTINCT CHARACTERISTICS FROM THE α- AND β-SUBTYPES

R. A. Bond, D. R. Blue & D. E. Clarke*, Department of Pharmacology, University of Houston, Houston, Texas 77004, U.S.A.

Experiments were done to characterize a putative adrenoceptor which is resistant to alpha- and beta-adrenoceptor antagonists and which functions to inhibit longitudinal muscle tension development in isolated segments of guinea-pig ileum (Bond et al, 1986; Bond & Clarke, 1987). Several phenylethylamine based agonists were investigated: BRL 37344 (Arch et al, 1984), isoprenaline, noradrenaline, adrenaline, and fenoterol. Propranolol and nadolol were tested as antagonists. Inhibition of the contractile response to histamine was measured.

Ileal segments from reserpine treated (5 mg/kg, i.p. for 18h) guinea-pigs were set-up in Krebs solution bubbled with 5% CO_2 and 95% O_2 to give a pH of 7.4. The Krebs solution was of the following composition (mM): NaCl 118, $CaCl_2$ 2.6, KCl 4.9, $NaHCO_3$ 25, NaH_2PO_4 1, $MgSO_4$ 1.2, glucose 11.7, choline 0.2 and also contained ascorbic acid (110 μM), cocaine (30 μM), corticosterone (30 μM), phentolamine (3 μM), and atropine (1 μM) to inhibit the auto-oxidation of amines, neuronal uptake, extraneuronal uptake, alpha-adrenoceptors, and muscarinic cholinergic receptors respectively. Repeated submaximal contractile responses to histamine (0.5 μM) were elicited every 5 or 10 min with 5 or 10 washes between additions.

Concentration dependent inhibitory responses were obtained with isoprenaline and BRL 37344 which were totally insensitive to beta-adrenoceptor blockade with concentrations of propranolol (5 μM) and nadolol (1 and 10 μM) which are between 100 and 1000 times their equilibrium dissociation constant for beta-adrenoceptors. These resistant responses to isoprenaline and BRL 37344 were antagonized by much higher concentrations of nadolol (30 μM to 1 mM) yielding apparent pA_2 values for nadolol of 4.40 (with isoprenaline as the agonist) and 4.70 (with BRL 37344 as the agonist), although the slopes of resulting Arunlakshana & Schild (1959) plots were steep (approximately 1.2). Similar apparent pA_2 values for nadolol at the putative adrenoceptor were obtained with noradrenaline (4.79), adrenaline (4.68), and fenoterol (4.38) as agonists. Nadolol, at 1 mM, the highest concentration used, failed to antagonize the inhibitory responses of papaverine toward histamine, indicating a selectivity of action. The order and relative potency of agonists at the putative adrenoceptor was: BRL 37344 (1) > isoprenaline (2.7) > noradrenaline (21) > adrenaline (44) > fenoterol (62).

The resistance to blockade by propranolol (5 μM), the apparent pA_2 values for nadolol (4.38 to 4.79), and the order and relative potency of agonists defines a receptor with apparently distinct characteristics from existing alpha- and beta-adrenoceptors. Whether the receptor is the same as the 'atypical' beta-adrenoceptor present on fat cells (Arch et al, 1984) and guinea-pig stomach fundus (Coleman et al, 1987) will be discussed in the light of current ways of defining effector receptors. (This work was supported by N.I.H. Grant GM 39621. We thank Dr. Arch for the supply of BRL 37344 and Dr. Aberg for the liberal supply of nadolol.)

Arch, J.R.S. et al (1984) Nature 309, 163-165
Arunlakshana, O. & Schild, H. O. (1959) Br. J. Pharmac. 15, 48-58
Bond, R. A. et al (1986) Naunyn-Schmiedeberg's Arch Pharmac. 334, 261-266
Bond, R.A. & Clarke, D.E. (1987) Br. J. Pharmac. 91, 683-686
Coleman, R.A. et al (1987) Br. J. Pharmac. 90, 40P

EVIDENCE THAT THE DOG PULMONARY ARTERY CONTAINS A HOMOGENOUS POPULATION OF α_1-ADRENOCEPTORS

J.R. Docherty* & R.R. Ruffolo[1]. Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2, Ireland, & [1]Department of Pharmacology, Smith Kline & French Laboratories, King of Prussia, PA, USA.

Flavahan & Vanhoutte (1986) have suggested that there may be two subtypes of alpha-1 adrenoceptor in vascular smooth muscle based on data obtained in rabbit and dog pulmonary arteries. In rabbit pulmonary artery, prazosin antagonised the contractions to clonidine with a pA_2 value 1 log unit higher than against methoxamine (Holck et al., 1983) and in dog pulmonary artery prazosin antagonised the contractions to phenylephrine with a pA_2 value 1 log unit higher than against methoxamine (Flavahan et al., 1987). In both these studies, a single agonist concentration-response curve was obtained per tissue in the absence or presence of antagonist drug. In a previous study employing two agonist concentration-response curves per tissue, no evidence was found for subtypes of alpha-1 adrenoceptor in rabbit pulmonary artery (Docherty, 1987). In the present study we examine the dog pulmonary artery.

Rings of dog pulmonary artery were suspended in organ baths at 37°C in Krebs-Henseleit solution containing cocaine (3 μM), corticosterone (30 μM) and propranolol (1 μM) for isometric tension recording. Cumulative concentration-response curves were obtained to phenylephrine (phe), methoxamine (met) or noradrenaline (NA), and were repeated 2 hrs later after 1 hr exposure to antagonist or vehicle. One tissue from each animal served as a vehicle control.

Phe, NA and met contracted the dog pulmonary artery with EC_{50} values of 1.9 μM (95% confidence limits of 1.17-3.09 μM), 1.12 μM (0.51-2.46 μM) and 28.2 μM (19.9-39.8 μM), respectively (n=17-23 tissues from 5-9 animals). Prazosin competitively antagonised contractions to phe and NA with pA_2 values of 9.56 and 9.32, respectively, with Schild plot slopes not significantly different from -1. However, due to the low potency of met, it was not possible to obtain maximum responses to this agonist in the presence of prazosin concentrations of 3 nM and above. However, since these prazosin concentrations did not reduce the maximum contraction to phe or NA, the maximum contraction to met in the presence of prazosin was calculated from the maximum response obtained in the first concentration-response curve (in the absence of prazosin). Under these conditions a pA_2 of 9.35 was obtained for prazosin against met.

Although we did not carry out experiments using the single concentration-response curve method, it was observed in comparing agonist potencies in our first concentration-response curves that agonist potency varied 3-10 fold in tissues from the same animal. Comparing NA potency in 3 tissues from each of 5 animals, the maximum difference in potencies was 4.07±1.55 fold (n=5).

In conclusion, we can find no evidence for subtypes of alpha-1 adrenoceptor in dog pulmonary artery when the classical two concentration-response curve method is used.

Docherty, J.R. (1987). Br. J. Pharmac. 91, 329P.
Flavahan, N.A. & Vanhoutte, P. (1986). Trends Pharmac. Sci. 7, 347-349.
Flavahan, N.A. et al. (1987). Br. J. Pharmac. 91, 331P.
Holck, M.I. et al. (1983). J. Cardiovasc. Pharmac., 5, 240-248.

CORRELATION OF CHANGES IN CGMP LEVELS WITH RELAXATIONS OF RAT ISOLATED AORTIC RINGS

K.J. Morrison* & D. Pollock, Department of Pharmacology, University of Glasgow, Glasgow, G12 8QQ, Scotland

Isolated blood vessels can be relaxed by acetylcholine (ACh), sodium nitroprusside (SNP) and an inhibitory factor (IF) extracted from bovine retractor penis (BRP) muscle (Bowman et al, 1981). The inhibitory effects of these substances can be demonstrated in isolated blood vessels in which the tone has been raised with noradrenaline (NA) or other agonists. The relaxant effect of ACh on blood vessels requires the presence of an intact endothelium (Furchgott & Zawadzki, 1980) but the inhibitory effects of IF or SNP are not dependent on the presence of an intact endothelium (Bowman et al, 1986).

Various studies have shown that inhibition of vascular smooth muscle tone by ACh and SNP is associated with stimulation of guanylate cyclase activity and increased levels of cGMP (Rapoport & Murad, 1983). Furthermore, IF-induced relaxation of the isolated BRP muscle is preceded by an increase in cGMP levels (Bowman & Drummond, 1984). This study therefore examined the ability of ACh, SNP and IF to relax rat isolated aortic rings with intact endothelia and precontracted with NA. In addition, the effects of these drugs on cGMP levels were examined in the same tissues.

Aortic rings (2-3 mm in length), prepared from the descending thoracic aorta of male Wistar rats (200-250 g) were suspended, with an initial resting tension of 2 g, between wire hooks in 25 ml organ baths, containing Krebs bicarbonate buffer, gassed with a mixture of 95% O_2/5% CO_2. Isometric tension was measured with Statham force displacement transducers and displayed on a Linseis recorder. In each tissue the tone was raised with NA (EC_{50} : 1.6×10^{-8} M) and, subsequently, at various times after adding the relaxant drug (ACh, SNP, IF), tissues were frozen in liquid nitrogen, then homogenized and extracted in trichloracetic acid (5% w/v). The cGMP content of an aliquot of each extract was determined by radioimmune assay (Amersham RIA Kit).

The endothelium-dependent vascular relaxation produced by ACh (10^{-5} M) was accompanied by an initial 10-fold increase in cGMP content (control:30.8 $\pm$ 6.2 $pmol g^{-1}$. ACh-treated:324.1 $\pm$ 80.4 $pmol g^{-1}$ mean $\pm$ s.e. mean, n = 9, $P<0.001$) but this raised level was not maintained throughout the relaxation. Elevated cGMP levels may be necessary for the development but not for the maintenance of ACh-induced relaxation. SNP (10^{-6} M) produced a 100-fold increase in cGMP levels (control:30.8 $pmol g^{-1}$. SNP-treated:3350.9 $\pm$ 435.3 $pmol g^{-1}$. n = 9, $P<0.001$). The time-course of this effect initially correlated with the endothelium-independent vascular relaxation produced by SNP but was not maintained throughout the relaxation. The development and perhaps the maintenance of SNP-induced vascular relaxation may be mediated by cGMP. Bovine IF (200 µl) produced only a partial relaxation, which was endothelium-independent. This effect of IF was not accompanied by any change in the levels of cGMP.

K.J.M. is an M.R.C. Scholar

Bowman, A. et al (1981) Br.J.Pharmac. 72, 365-372
Furchgott, R.F. & Zawadzki, J.V. (1980) Nature 288, 373-376
Bowman, A. et al (1986) Br.J.Pharmac. 87, 175-181
Rapoport, R.M. & Murad, F. (1983) J.Cyclic Nucleotide Protein Phosph. Res. 9, 281-196
Bowman, A. & Drummond, A.H. (1984) Br.J.Pharmac. 81, 665-674

TWO POPULATIONS OF MINIATURE END-PLATE CURRENTS IN SNAKE SKELETAL MUSCLE

C. Prior*, T. Searl & I.G. Marshall. Department of Physiology & Pharmacology, University of Strathclyde, Glasgow.

Previous reports suggest the existence of a separate population of small miniature end-plate currents (MEPCS) in frog (Bevan, 1976) and rat (Cooke & Quastel, 1973) muscle which is revealed following intense nerve stimulation. As a part of an investigation into the actions of vesamicol, an inhibitor of vesicular acetylcholine storage, we observed the presence of small MEPCS in snake muscle. To gain insight into the nature of these small MEPCS we have studied the effect of nerve stimulation on MEPCS recorded from snake muscle.

MEPCS were recorded from the garter snake costocutaneous muscle voltage-clamped at -110mV. Following a 5min period during which control MEPCS were recorded, the motor nerve was stimulated for 5min (2, 5 or 10Hz). Immediately after this a second 5min record of MEPCS was made. Experiments were performed at each of three calcium ion concentrations (1.5, 2 and 4mM). Amplitude histograms were constructed for all MEPC data to allow comparison of results.

Nerve stimulation had no effect on MEPC time course at any frequency or [Ca] studied. However, mean MEPC amplitude decreased following stimulation in both a frequency and [Ca]-dependent manner. Thus with 1.5mM [Ca] post-5Hz MEPC amplitude was unchanged while in 4mM [Ca] post-5Hz MEPC amplitude was reduced to 79 ± 2% (n=5, $P<0.05$ paired t-test) of the pre-stimulation value. This decrease in mean MEPC amplitude was not due to a shift of the entire population to lower amplitudes but to the appearance of a new population of small MEPCS. Subtraction of the pre-stimulation MEPC amplitude relative frequency histogram from the post-stimulation histogram revealed two peaks which were individually analysed for relative proportion and mean MEPC amplitude. Thus the 20% reduction in MEPC amplitude seen at 5Hz/4mM [Ca] can be attributed to the appearance of a second population of MEPCS with mean amplitude 58 ± 3% (n=5) of control and which made up 44 ± 4% (n=5) of all the MEPCS seen during the post-5Hz recording period. No change in the mean amplitude of the large MEPCS was seen post-stimulation.

The appearance of the small MEPCS following nerve-stimulation was transient. During the first minute post-5Hz (4.0mM [Ca]) MEPC amplitude was 71 ± 1% of control and the small MEPCS made up 56 ± 3% of the total (means of 2 cells). In the fifth minute following stimulation these values were 88 ± 5% and 29 ± 10% respectively. However there was no change in the mean MEPC amplitude in either of the individual populations with time. Full recovery from the effects of 5Hz nerve-stimulation for 5min with 4mM [Ca] took approximately 20min.

The exact nature of these small MEPCS remains unclear. Their appearance is related to the amount of evoked transmitter released since they can be elicited by increasing the number of quanta released by the nerve terminal per minute. The most noticable difference between our observations and those made previously is the magnitude of the effect. Even at relatively moderate rates of nerve stimulation (5Hz/2mM [Ca]) 18 ± 4% (n=7) of the 5min post-stimulation MEPCS were from the small population. Thus the snake preparation may prove to be an invaluable tool for the investigation of so called "small-mode" MEPCS.

Bevan, S. (1976). J. Physiol. 258, 145-155.
Cooke, D.J. & Quastel, D.M.J. (1973). J. Physiol. 228, 377-405.

Supported by a grant from the Wellcome Trust.

INTERACTIONS BETWEEN CHOLINERGIC AND NORADRENERGIC NERVES IN THE RAT ISOLATED ATRIA

S.J. Boyle* & D. Pollock, Department of Pharmacology, University of Glasgow, Glasgow, G12 8QQ, Scotland

Sympathetic nerve terminals in the rat heart have muscarinic cholinoceptors and α_2-adrenoceptors, which when activated by the appropriate agonists, inhibit noradrenaline (NA) release (Langer, 1977). This was confirmed in experiments in which field stimulation of isolated atria produced a positive inotropic post-stimulation response (IPSR), that was inhibited by guanethidine (10^{-6} M) and potentiated by yohimbine (10^{-7} M) and atropine (10^{-6} M) (Boyle & Pollock, 1987). Surprisingly, the IPSR was unaffected by clonidine, which did inhibit field stimulation-induced ^3H overflow in atria previously incubated with (^3H)-NA. This anomaly could be explained if there are presynaptic α-adrenoceptors on the cholinergic nerve terminals. In such circumstances clonidine might inhibit (^3H)-NA release but would also inhibit ACh release, so that the inhibitory effect of neurally-released ACh on presynaptic muscarinic cholinoceptors on the sympathetic nerves would be reduced and thus the IPSR would be unaffected.

This study investigated the effects of drugs on ^3H and ^{14}C overflow in rat isolated atria, previously incubated in (^3H)-NA and (^{14}C)-choline, to determine whether there are α_2-adrenoceptors on the cholinergic nerve terminals as well as on the sympathetic nerve terminals.

Paired, spontaneously beating atria from male Wistar rats (200-250 g) were incubated in (^3H)-NA (500 nM, 43 Ci $mmol^{-1}$, 30 min, 37°C) and (^{14}C)-choline (500 nM, 43 Ci $mmol^{-1}$, 60 min, 37°C) and inserted into silver ring electrodes in 2 ml organ baths, containing Krebs bicarbonate buffer, gassed with O_2 95%/CO_2 5% and containing eserine (10^{-6} M) and hemicholinium (10^{-5} M). Atria were stimulated (525 pulses of 1 ms duration, 5 Hz at supramaximal voltage). The Krebs buffer was collected during and between stimulations and the radio-activity in each sample was counted.

^3H and ^{14}C overflow was inhibited by ACh (10^{-5} M), clonidine (10^{-5} M) and tetrodotoxin (5×10^{-6} M) and potentiated by atropine (10^{-5} M) and yohimbine (10^{-5} M). These results confirm the presence of α_2-adrenoceptors and muscarinic cholinoceptors on the sympathetic nerve terminals and suggest that similar receptors are present on the cholinergic nerve terminals, so that neurally-released ACh and NA participate not only in auto-inhibition of transmitter release but also in mutual cross inhibition, with ACh inhibiting NA release and NA inhibiting ACh release.

S.J.B. is a University of Glasgow Postgraduate Scholar

Langer, S.Z. (1977) Br.J.Pharmac. 60, 481-497
Boyle, S.J. & Pollock, D. (1987). J.Physiol. 386, 53P

N-METHYL-HYDROXYLAMINE, A GUANYLATE CYCLASE INHIBITOR, BLOCKS NANC RELAXATIONS OF THE MOUSE ANOCOCCYGEUS

A. Gibson, Department of Pharmacology, Kings College London, Chelsea Campus, Manresa Road, London SW3 6LX.

It has been shown recently that non-adrenergic, non-cholinergic (NANC) relaxations of some smooth muscles (bovine retractor penis, opossum lower oesophageal sphincter) may involve activation of guanylate cyclase by the NANC transmitter (Bowman & Drummond, 1984 ; Torphy et al., 1986). The object of the present study was to investigate the possibility that NANC transmission in the mouse anococcygeus muscle might also fall within this group, by observing the effects of N-methyl-hydroxylamine (NMH), a known inhibitor of guanylate cyclase (Deguchi et al., 1978), on relaxations induced by field stimulation or drugs.

Anococcygeus muscles were isolated from male mice (LACA strain) and set up for the recording of isometric tension responses. Biphasic NANC relaxations to 60 s trains of field stimulation (10 Hz) were obtained as described previously (Gibson & Yu, 1983). Motor sympathetic responses were prevented by preincubation with guanethidine (30 μM ; 10 min) and by inclusion of phentolamine (1 μM) in the Krebs bathing medium throughout the experiment. Muscle tone was raised with carbachol (50 μM).

NMH (1 - 5 mM) by itself produced transient, dose-related relaxations of carbachol induced tone, and at the same time inhibited the further relaxations induced by NANC stimulation. In the continued presence of NMH,tone returned to its original level within 10 min, although inhibition of NANC relaxations persisted. Both phases of the NANC response were inhibited to the same extent ; 2 mM NMH reduced the first phase by 49 ± 5% and the second by 60 ± 7% (n = 8 in both cases). The inhibition by NMH was easily reversed by washout. Conversely, 2 mM NMH did not inhibit, but rather potentiated, contractions of the anococcygeus induced by field stimulation (30 Hz ; 10 s) in the absence of sympatholytic drugs. It is likely that this potentiation resulted from removal of the NANC component of the response to field stimulation since NMH had no effect on contractions elicited by submaximal concentrations of noradrenaline (5 μM).

Dose-related relaxations were produced by sodium nitroprusside (SNP ; 0.01 - 1 μM) vasoactive intestinal peptide (VIP ; 0.1 - 2 μM), papaverine (1 - 40 μM), 3-isobutyl-1-methylxanthine (IBMX ; 1 - 40 μM) and adenosine 5'-triphosphate (ATP ; 0.2 - 10 mM). The effect of NMH was studied on concentrations of these relaxants which reduced tone by between 40 - 60%. 2 mM NMH inhibited relaxations to 0.1 μM SNP by 69 ± 6% (n = 6) but had no significant effect on relaxations to VIP (1 μM), papaverine (10 μM), or IBMX (10 μM). Relaxations to ATP (2 mM) were slightly potentiated, by 19 ± 2% (n = 6). A Schild plot of NMH (1 - 5 mM) against SNP yielded a pA_2 value of 3.08 (slope = 0.87).

In conclusion, NMH is an effective inhibitor of NANC transmission in the mouse anococcygeus ; that guanylate cyclase inhibition underlies this effect is indicated by the selective inhibition of responses to SNP, a known activator of guanylate cyclase (Ignarro & Kadowitz, 1985). Since relaxations to VIP and ATP were not inhibited by NMH, it is unlikely that either acts as the NANC transmitter in this tissue.

Bowman, A. & Drummond, A.H. (1984) Br. J. Pharmac. 81, 665 - 674.
Deguchi, T., Saito, M., & Kono, M. (1978) Biochim. Biophys. Acta 544, 8 - 19.
Gibson, A. & Yu, O. (1983) Br. J. Pharmac. 79, 611 - 616.
Ignarro, L.J. & Kadowitz, P.J. (1985) Ann. Rev. Pharmac. Toxicol. 25, 171 - 191.
Torphy, T.J., Fine, C.F., Burman, M., Barnette, M.S., & Ormsbee, H.S. (1986) Am. J. Physiol. 251, G786 - G793.

THE RELEVANCE OF DETERMINATION OF LYMPHOCYTE β_2-ADRENOCEPTORS AS INDEX FOR β-ADRENOCEPTORS IN OTHER HUMAN TISSUES

O.-E.Brodde*, M.C.Michel, N.Nohlen, X.L.Wang and H.-R.Zerkowski, Biochem. Res.Lab., Med. Klinik & Poliklinik, University of Essen, D-4300 Essen, Fed. Rep. Germany

Determination of β_2-adrenoceptors (AR) in circulating lymphocytes is a frequently used model to study alterations of β-AR function in man (Brodde et al., 1987). To find out whether it is generally valid to extrapolate from changes in lymphocyte β-AR to β-AR alterations in other human tissues, in the present study we compared lymphocyte β-AR densities with β-AR densities in the corresponding myometrial and right atrial membranes, respectively.

Myometrial strips were obtained from 36 women aged 15-41 years undergoing cesarean section. Six of them were on tocolytic therapy for at least 5d before operation (consisting of continuous infusion of 0.32 µg/min hexoprenaline accompanied by infusion (100 µg/min) or oral treatment with 3x50 mg/d metoprolol) to prevent preterm labor. Myometrial β-AR density was assessed by (-)-[^{125}I]iodopindolol (IPIN) binding at 6-8 concentrations of IPIN ranging from 20-300pM. Non-specific binding was defined as binding in the presence of 1µM (±)-CGP 12177. Right atrial appendages were obtained from 44 patients aged 44-73 years (NYHA class I-II) undergoing coronary artery bypass grafting. The patients were divided into 5 age-matched groups according to the chronic pretreatment with β-AR antagonists: I (n=10) were on sotalol or propranolol, II (n=10) on metoprolol, III (n=10) on atenolol and IV (n=4) on pindolol treatment. Ten patients not treated with β-AR antagonists were taken as controls. Atrial β-AR density, the relative amount of atrial β_1- and β_2-AR and β-AR density in the corresponding lymphocytes were assessed by (-)-[^{125}I]iodocyanopindolol (ICYP) binding as recently described (Michel et al., 1986).

The mean number of myometrial β-AR (85% β_2-, 15% β_1-AR) of untreated women (16.6 ± 1.6 fmol IPIN bound/mg protein, n=30) was significantly higher than in hexoprenaline treated women (6.1±1.4 fmol/mg protein, n=6, $P<0.01$). The same held true for lymphocyte β_2-AR density (1412±105 in untreated vs. 454±85 ICYP binding sites/cell in hexoprenaline treated women, $P<0.001$). When β-AR densities in myometria and lymphocytes obtained from the same patients were compared, a significant positive correlation was obtained ($r=0.611$, n=25, $P<0.005$).
The mean number of β-AR in atrial membranes (70% β_1-, 30% β_2-AR) in the control group (i.e. patients not treated with β-AR antagonists) amounted to 70.0±8.0 fmol ICYP bound/mg protein. With the exception of the pindolol group β-AR density was in all groups of patients treated with the different β-AR antagonists significantly higher than in control. Detailed analysis of these increases revealed that all β-AR antagonists significantly increased atrial β_1-AR density; in contrast, only sotalol or propranolol increased atrial β_2-AR density, while metoprolol or atenolol did not affect it. Similarly, β_2-AR density in the corresponding lymphocytes was only increased in the sotalol/propranolol group, while it was nearly identical to control in the metoprolol or atenolol group. Pindolol exhibited a differential pattern of β-AR regulation: it increased atrial β_1-AR density, but decreased atrial and lymphocyte β_2-AR density. Accordingly, plotting mean lymphocyte β_2-AR density of each group against mean atrial β_2-AR density of each group resulted in a significant positive correlation ($r=0.903$, n=5, $P<0.05$), whereas atrial β_1-AR densities were not at all related to lymphocyte β_2-AR densities.

It is concluded that changes in lymphocyte β-AR can be taken as representative for β-AR changes in other human tissues only, when these changes are caused by non-selective β-AR agents.However, if β_1- or β_2-AR selective drugs are involved lymphocyte β-AR mirror precisely β_2-AR changes, but only slightly β_1-AR changes, if at all.

Brodde, O.-E. et al. (1987). Trends Pharmacol. Sci. 8: 403-407.
Michel, M.C. et al. (1986). J. Hypertension 4 (Suppl. 6): S215-S218.

28P

FACILITATORY α- AND ß-ADRENOCEPTORS ON THE RAT PHRENIC NERVE

Wessler, I., Anschütz, S. & Ladwein, E. (introduced by E. Muscholl), Department of Pharmacology, University of Mainz, F.R.G.

We have previously reported that after labelling of neuronal transmitter stores the release of [^{3}H]-acetylcholine (ACh) from the rat phrenic nerve can be measured in the absence of cholinesterase inhibitors (Wessler & Kilbinger, 1986). Using this radiolabelling technique evidence was presented that transmitter release from the motor nerve is under the control of presynaptic nicotine and muscarine receptors (Wessler et al., 1986; 1987). A modulatory role of sympathomimetic amines on transmitter release from the motor nerve has been postulated from results of electrophysiological and functional studies (Bowman, 1981). The present experiments were designed to investigate the effects of noradrenaline (NA), isoprenaline and α-methylnoradrenaline (MNA) on ACh release from the phrenic nerve.

Endplate preparations of the left hemidiaphragm of Sprague-Dawley rats were incubated (40 min) with 1 µM [^{3}H]-choline (10 µCi), and labelling of neuronal transmitter stores was carried out during a nerve stimulation at 1 Hz (0.2 msec impulse duration, 8 volts). After a subsequent 60 min washout tritium efflux was measured in 2 min samples. Release of ACh was elicited by two periods (S1, S2) of electrical nerve stimulation, 200 pulses at 10 Hz each. S1 was regarded as control and the substances were added before S2.

NA (1 µM) and isoprenaline (0.1 µM) enhanced the stimulated transmitter release by 112 $\pm$ 19% (n=8) and 106 $\pm$ 21% (n=7) respectively. Pretreatment with propranolol (0.1 µM) or atenolol (0.3 µM) prevented the facilitatory effect of both amines. Isoprenaline lost its facilitatory effect when the exposure time was increased from 16 to 24 min. MNA (10 µM) also enhanced (35 $\pm$ 11%, n=8) the stimulated ACh release. However, this effect could be blocked only by a combination of propranolol and yohimbine (10 µM). Immediately after their application both NA (27 $\pm$ 3%, n=10) or MNA (23 $\pm$ 4%, n=8) enhanced the basal tritium outflow which might indicate an increase in the spontaneous transmitter release. This effect disappeared after an exposure time of 10 - 12 min and was abolished only by a combined pretreatment with propranolol and yohimbine.

It is concluded that sympathomimetic amines enhance the stimulated transmitter release mainly by stimulation of presynatic ß-adrenoceptors whereas α-adrenoceptors play only a minor role in the modulation of the stimulated transmitter release. During a continued exposure to agonists the presynaptic ß-adrenoceptors desensitize within a time interval of several minutes and are probably of the ß1-subtype. Additionally, it is proposed that spontaneous transmitter release can be enhanced by stimulation of α- or ß-adrenoceptors.

Supported by the Deutsche Forschungsgemeinschaft.

Bowman, W.C. (1981). Handbook of Experimental Pharmacology 54, 47-128.
Wessler, I. & Kilbinger, H. (1986). Naunyn-Schmiedebergs Arch. Pharmacol. 334, 357-364.
Wessler, I. et al., (1986). Naunyn-Schmiedebergs Arch. Pharmacol. 334, 357-364.
Wessler, I. et al., (1987). Naunyn-Schmiedebergs Arch. Pharmacol. 335, 605-612.

ATP RECEPTORS IN THE RAT VAS DEFERENS

M.A.K.Lafi* & L.D.Leake, School of Biological Sciences, Portsmouth Polytechnic, King Henry I St., Portsmouth PO1 2DY, Hants., U.K.

Although the primary transmitter in the vas deferens is thought to be noradrenaline (NA) there is evidence that ATP acts as a co-transmitter with NA at this neuromuscular junction (Sneddon & Westfall, 1981; Meldrum & Burnstock,1983). In the guinea-pig pre- (P1) and postsynaptic (P2) receptors are involved (Sneddon & Burnstock, 1984; Sneddon et al.,1984). In this study we investigate purinergic receptors in the rat.

Vasa deferentia from Sprague-Dawley rats (200-300g) were set up in Mg^{2+}-free Tyrodes bubbled with 95% O_2:5% CO_2. Some rats were chronically denervated by i.p.injection.of 25mg guanethidine/kg/day 5 days a week for 6 weeks. Controls received 0.85% saline injections.

In control and denervated vasa ATP (100μM) induced a biphasic contraction with an initial fast component followed by a slower one, ADP gave a small tonic contraction, α,β-methyleneATP (αβmeATP)(0.5μM) a fast one only and adenosine no response even at 200μM. In denervated preparations the ATP response was reduced by 0.1μM phentolamine; reduced, but not abolished, by desensitization with repeated doses of αβmeATP and further reduced by addition of phentolamine before the αβmeATP. In controls ATP (100μM) did not alter tonic responses to 1μM NA although NA markedly potentiated the fast ATP response. In denervated vasa ATP slightly inhibited responses to 0.1μM NA while NA strongly enhanced the ATP response. Adenosine (100μM) and NA did not modify each other. In the control prostatic end, unlike the epididymal end, transmural (TM) stimulation evoked contractions which were only slightly inhibited in the presence of atropine and prazosin. Addition of αβmeATP enhanced these contractions and induced a tonic contraction which disappeared when the dose was repeated. Subsequent addition of ATP (100μM) produced a tonic response but inhibited evoked contractions. In the denervated prostatic end, in the presence of eserine, TM-evoked contractions were abolished by an acute dose of guanethidine but restored by αβmeATP and subsequently abolished by atropine. In the presence of αβmeATP and eserine, guanethdine had no effect on evoked contractions.

These results suggest that ATP, αβmeATP and ADP produce contractions in the rat vas via a direct action on postsynaptic P2 receptors; and that ATP and αβmeATP must exert their actions via two different P2 receptor sub-types. NA must interact postsynaptically with ATP to result in enhanced responses to ATP. The importance of a cholinergic mechanism is emphasised, particularly at the prostatic end, and an interaction of αβmeATP with this mechanism is postulated.

Meldrum, L.A. & Burnstock, G. (1983) Eur.J.Pharmacol. 92, 161-163.
Sneddon, P. & Burnstock, G. (1984) Eur.J.Pharmacol. 100, 85-90.
Sneddon, P. & Westfall, D.P. (1984) J.Physiol. 347, 561-580.
Sneddon, P., Meldrum,L.A. & Burnstock G. (1984) Eur.J.Pharmacol. 105, 293-299.

* Present address : Dept. Pharmacology, Military Medical College, Zufuraniya, Baghdad, Iraq.

THE DISPOSITION OF VITAMIN K_1 IN THE RABBIT

S. Cholerton, M.J. Winn*, B.K. Park, Department of Pharmacology and Therapeutics, University of Liverpool, New Medical Building, P.O. Box 147, Liverpool, L69 3BX.

In an attempt to rationalize the requirement for large and repeated doses of vitamin K_1 during coumarin poisoning, we have measured the concentration of vitamin K_1 in plasma, liver homogenate and liver microsomes in the absence and presence of brodifacoum.

Male, New Zealand White rabbits (weights 2-3 kg) were used. Twenty-four hours after the administration of either Brodifacoum (10mg kg^{-1} i.v.; n = 6) or its vehicle (polyethylene glycol 200; 0.5ml kg^{-1} i.v.; n = 6), vitamin K_1 (10mg kg^{-1} ; 1ml kg^{-1} i.v.) was given. Blood samples (5ml) were collected from the right marginal ear vein. Plasma was assayed by HPLC (Wilson & Park 1983; Hart et al., 1985). Further blood samples (1ml) were obtained for the measurement of prothrombin complex activity and the determination of clotting factor synthesis (Breckenridge et al., 1985). To investigate the concentrations of vitamin K_1 in rabbit liver, livers were removed either 3h or 24h after vitamin K_1. Liver and microsomal concentrations of vitamin K_1 were determined by HPLC (Hart et al. 1985; Cholerton & Park 1986).

After intravenous administration of vitamin K_1, plasma concentrations of the vitamin fell triexponentially (Hart et al., 1984). There were no significant differences between the two groups over the first 24h after vitamin K_1, but between 24h and 77h plasma levels of vitamin K_1 were significantly ($p < 0.05$) lower in anticoagulated rabbits than in control rabbits. Whilst the administration of vitamin K_1 initially promoted clotting factor synthesis, clotting factor synthesis had ceased 3-6h after the vitamin, despite plasma concentrations (7.7 $\pm$ 4.1 $\mu g\ ml^{-1}$) 1000-fold greater than normal at this time (8.2 $\pm$ 1.0ng ml^{-1}).

In whole liver the resting concentration of vitamin K_1 was 127.7 $\pm$ 44.3 ng g^{-1}. Three hours after vitamin K_1 there was a similar rise in hepatic concentrations of the vitamin in rabbits given brodifacoum (46.6 $\pm$ 4.3$\mu g\ g^{-1}$) and rabbits given vehicle (32.8 $\pm$ 6.4 $\mu g\ g^{-1}$). Furthermore, there were no significant differences between the microsomal concentrations of vitamin K_1 in rabbits given brodifacoum (4.00 $\pm$ 2.28 $\mu g.mg\ protein^{-1}$) and rabbits given vehicle (2.65 $\pm$ 1.01 $\mu g.mg\ protein^{-1}$). After 24h, whole liver concentrations had fallen markedly, yet remained significantly ($p < 0.05$) above resting levels (2.7 $\pm$ 0.9$\mu g\ g^{-1}$ rabbits given brodifacoum; 4.5 $\pm$ 0.92$\mu g\ g^{-1}$ rabbits given vehicle). Thus it appears that the need for repeated administration of vitamin K_1 during coumarin poisoning is due to a greatly increased requirement for the vitamin, and to the rapid clearance of the vitamin from both plasma and liver.

MJW is a Ward-Blenkinsop research fellow; BKP is a Wellcome Senior Lecturer.

Breckenridge, et al., (1985) Br. J. Pharmac. 84, 81-91.
Cholerton, S. & Park, B.K. (1986) J. Chromatogr. 375, 147-153.
Hart, J.A.D. et al., (1984) Biochem. Pharmac. 33, 3013-3019.
Hart, J.P. et al., (1985) Analyst 110, 1181-1184.
Wilson, A.C. & Park B.K. (1982) J. Chromatogr. 277, 292-299.

PRIMAQUINE ELIMINATION PROFILE IN URINE DETERMINED BY A NEW RAPID AND SENSITIVE ELISA METHOD

F.J. Rowell* & V. Rowell, Department of Pharmaceutical Chemistry, Sunderland Polytechnic, Sunderland, SR2 7EE.

Primaquine is used against the exo-erythrocytic stages of Plasmodium vivax and Plasmodium ovale (Strube, 1975). In common with other antimalarial drugs simple yet sensitive and specific assays are required to measure levels in biological fluids in order to detect the drug in conjunction with screens for in vitro drug suspectibility studies and to determine concentrations in conjunction with pharmacokinetic studies. Such assays should suit the skills and facilities likely to be present in areas where malaria is endemic. A Enzyme-linked immunosorbent assay (ELISA) has therefore been developed and used to monitor levels of primaquine-immunoreactivity in the urine of a volunteer receiving a single oral dose(22.5mg base) in order to assess the practicability and specificity of the assay.

The immunogen was prepared by linking primaquine to keyhole limpet haemocyanin through its primary amino group. The antiserum was produced in sheep (Al-Abdulla *et al* 1987). Primaquine was directly linked to porcine thyroglobulin and the conjugate used in a competitive ELISA following procedures described for quinine (Rowell & Rowell, 1987). The ELISA is specific for primaquine exhibiting no cross-reactivity with other anti-malarials or common drugs at concentrations less than 10^{-4}M. Urine at volumes less than 5 µl do not interfere in the assay which has a limit of sensitivity of 5ng/ml using this sample volume. Total assay time for a 96 well plate using triplicate standards (5) and samples (27) is 90 min. The coefficient of variation is 11.2-13.2% and the recovery 98.0-98.6% for urine samples of concentrations 50-500ng/ml. The assay shows no significant cross-reactivity to proposed quinoline-ring metabolites (Fletcher *et al* 1977) but shows equal cross-reactivity between the major metabilite, carboxyprimaquine (Mihaly *et al* 1984) and the parent drug. Primaquine was separated from carboxyprimaquine using a modified extraction procedure to that described by Mihaly *et al* (1984) and Al-Abdulla (1987) and the urine and extracted components were assayed by the immunoassay.

The results indicate that primaquine-immunoreactive compounds can be detected in the urine of the volunteer for 75 hours after dosing. The urinary elimination profile is polyphasic exhibiting peaks after 3.5, 6, 9, 11 and 37 hours. Analysis of the urinary extracts indicates that the initial peak is due to primaquine and the subsequent peaks are due to a polar non-extractable metabolite(s) which has a primaquine-like structure.

Acknowledgement This investigation received financial support from the UNDP/ World Bank/WHO Special Programme for Research and Training in Tropical Diseases.

Al-Abdulla, J.H., Sidki, A.M., Landon, J. & Rowell, F.J. (1987). Clin. Chim. Acta. Accepted for publication.
Fletcher, K.A., Canning, M.V. & Gilles, H.M. (1977) Trans. Roy. Soc. Trop. Med. Hyg., 71, 111.
Mihaly, G.W., Ward, S.A., Edwards, G., Orme, M. L'E & Breckenridge, A.M. (1984) Br. J. Clin. Pharmac 17, 441-446.
Rowell, V. & Rowell, F.J. (1987) Analyst. 112, 1437-1439 (1987).
Strube, R.E. (1975) J. Trop. Med. Hyg. 78, 171-186.

HEPATIC EFFECTS OF CINNAMYL ANTHRANILATE RESEMBLE THOSE OF A PEROXISOME PROLIFERATOR IN MOUSE, BUT NOT RAT

A. Viswalingam,* J. Caldwell, S. Fournel,+ L. Schladt++ & F. Oesch,++ Department of Pharmacology and Toxicology, St. Mary's Hospital Medical School, London W2 1PG. +Centre de Medicament, University of Nancy, France. ++Institute of Toxicology, University of Mainz, FRG.

The synthetic ester cinnamyl anthranilate (CA) was formerly used as a food flavour until its withdrawal due to murine, but not rat, hepatocarcinogenicity. We have previously shown CA to be an hepatic enzyme inducer in mice (Caldwell *et al.*, 1985) and the present report concerns a more detailed examination of its effects in rats and mice. Following 3 daily doses of 100 or 1000 mg/kg to male CD-1 mice, there occurred a dose-related hepatomegaly and induction of microsomal cytochrome P-450, the Soret band of which was at 451.5-452 nm. The microsomal N-demethylation of aminopyrine was reduced but there was an enhancement of lauric acid 11- and 12-hydroxylation. Microsomal bilirubin glucuronidation was doubled, but microsomal epoxide hydrolase activity towards styrene oxide was unaltered. However, cytosolic epoxide hydrolase activity towards *trans*-stilbene oxide was increased. There occurred a 5 fold increase in CN^--insensitive palmitoyl CoA oxidation, a peroxisomal marker enzyme. In contrast, the administration of CA at the same doses to male F-344 rats had no systematic effects upon any of these parameters of liver function.

Table 1. Hepatic effects of CA in rats and mice

	% change relative to control			
	Mouse		Rat	
Dose (mg/kg day x 3)	100	1000	100	1000
Relative liver weight %)	125^{++++}	155^{++++}	110	124^{++++}
CN^--insensitive palmitoyl CoA oxidation*	424^{++++}	501^{++++}	101	191^{++++}
Microsomal protein (mg/g liver)	131	146^{+}	89	136
Microsomal cytochrome P-450 (mol/mg protein)	155^{+}	212^{+++}	84	104
Aminopyrine N-demethylation*	31	55	82	150
Lauric acid 11- and 12-hydroxylation*	1560^{++++}	1720^{++++}	43	86
Bilirubin glucuronidation*	137	190	127^{+}	140^{++++}
Microsomal epoxide hydrolase*	69	81	64	122
Cytosolic epoxide hydrolase**	262^{++}	322^{+++}	120	228^{++++}

* nmol/min/mg protein **pmol/min/mg protein; $n \geq 5$.

$^{+}$ $p<0.05$ $^{++}$ $p<0.02$ $^{+++}$ $p<0.01$ $^{++++}$ $p<0.001$

These data show CA to cause, in mice but not rats, a pattern of enzyme induction, of the type produced by hypolipidaemic drugs and plasticizers, which is linked with peroxisomal proliferation (Rhodes *et al.*, 1986; Schladt *et al.*, 1986). In view of the known association in rats and mice, but not other species, between such patterns of enzyme induction in subchronic tests and the appearance of hepatocellular carcinoma (Rao & Reddy, 1987) in long term studies, this finding may aid in interpreting the significance of the murine hepatocarcinogenicity of CA for the safe use of this compound in the human diet.

Supported by grants from MAFF (Project 513) and FEMA

Caldwell, J. *et al.* (1985) Fd. Chem. Toxicol., 23, 559.
Rao, M.S. & Reddy, J.K. (1987) Carcinogenesis, 8, 631.
Rhodes, C. *et al.* (1986) Envir. Hlth. Persp., 65, 299.
Schladt, L. *et al.* (1986) Biochem. Pharmacol., 35, 3309.

CHARACTERISATION OF H_1 AND H_2-RECEPTOR FUNCTION IN THE TRACHEAL CIRCULATION OF SHEEP

S.E. Webber*, R.O. Salonen & J.G. Widdicombe, Department of Physiology, St. George's Hospital Medical School, London SW17 0RE.

The effects of histamine, the specific H_1-agonist SKF 71482 and the H_2-agonist dimaprit were examined on tracheal vascular resistance and smooth muscle tone in sheep.

The cranial tracheal arteries were perfused bilaterally in sheep anaesthetised with 20 $mg.kg^{-1}$ pentobarbitone, paralysed with 1 $mg.kg^{-1}$ gallamine triethiodide and artificially ventilated. Perfusion was via segments of common carotid artery, with arteries to skeletal muscle and glandular tissues tied off. Perfusion was at constant flow, usually 6-10 $ml.min^{-1}$, and inflow pressure was measured and divided by flow to give vascular resistance. Tracheal smooth muscle tone was assessed from changes in the external diameter of the upper trachea. All drugs were injected directly into the catheter supplying the tracheal vascular bed.

Histamine produced either a triphasic vascular change (early dilation then constriction followed by late dilation) or constriction alone. SKF 71482 always produced a biphasic change (dilation followed by constriction). Dimaprit dilated the vasculature. All effects of the agonists were dose-dependent. The pD_2 values and maximum responses are shown in Table 1.

Table 1 pD_2 Values and maximum responses for the effects of histamine and specific H_1 and H_2-receptor agonists on tracheal vascular resistance

Agonist	Response	Phase	pD2	Maximum (% change)
Histamine	Triphasic	1. Dilation	8.1 ± 0.4 (4)	-16.1 ± 4.1 (4)
		2. Constriction	ND	ND
		3. Dilation	7.8 ± 0.3 (4)	-30.8 ± 6.4 (4)
	Constriction only	1. Constriction	7.8 ± 0.2 (3)	+54.6 ± 7.2 (3)
SKF 71482	Biphasic	1. Dilation	7.1 ± 0.3 (5)	-23.6 ± 0.8 (5)
		2. Constriction	6.9 ± 0.4 (5)	+14.2 ± 1.8 (5)
Dimaprit	Dilation only	1. Dilation	6.4 ± 0.2 (6)	-18.4 ± 3.8 (6)

The late dilation, but no other vascular responses, produced by histamine in some sheep was significantly antagonised ($p < 0.05$, paired t-test) by bilateral cervical vagotomy. Both the constriction and early dilation due to histamine and SKF 71482 were significantly antagonised by mepyramine (50 mg, i.v.), indicating H_1-receptor mediated effects. Cimetidine (250 mg, i.v.) had no significant effect on the constriction or early dilation due to histamine, suggesting a lack of involvement of H_2-receptors. The dilator effect of dimaprit was significantly antagonised by cimetidine, suggesting a mediation by H_2-receptors. Histamine and SKF 71482 both contracted the tracheal smooth muscle and this effect was significantly antagonised by mepyramine. Dimaprit had no effect on tracheal smooth muscle tone.

We conclude that various tracheal vessels have dilator H_2-receptors and constrictor and dilator H_1-receptors, and that the smooth muscle has contractile H_1-receptors.

34P

HISTAMINE SECRETION FROM MAST CELLS STIMULATED WITH SODIUM ORTHOVANADATE

Mariam Al-Laith* & F.L. Pearce, Department of Chemistry, University College London, 20 Gordon Street, London WC1H 0AJ.

Sodium orthovanadate has been widely used as an experimental probe in a number of contractile systems, particularly cardiac muscle (Jandhyla & Hom, 1983; Akera et al, 1983). The anion has a range of pharmacological activities but most relevantly stimulates adenylate cyclase, inhibits membrane sodium-potassium and calcium ATPases, promotes the mobilisation of intracellular calcium stores and stimulates influx of calcium through voltage-dependent or independent channels. Since histamine release is a calcium-dependent secretory process (Pearce, 1982), and given the close analogy between the mechanisms involved in stimulus-secretion and stimulus-contraction coupling, we have now examined the effect of vanadate on isolated mast cells.

Sodium orthovanadate (1-10 mM) produced a dose-dependent secretion of histamine from rat peritoneal mast cells. The maximum release amounted to *ca* 60% of the total cellular content of the amine. The process was slow, exhibited a lag-phase of about 30 min and required 60-90 min for completion. The effect was strongly dependent on pH and increased progressively over the range 6.0 to 8.5. In contrast, other pharmacological and immunological ligands showed a maximal release at physiological pH. The release induced by vanadate was highly calcium-dependent and optimal secretion was observed at a concentration of the divalent cation of 1 mM. Equimolar concentrations of strontium could substitute for calcium. The response was non-cytotoxic and was inhibited by extremes of temperature and by metabolic blockers. In contrast to immunological stimuli, histamine release induced by vanadate was unaffected by lanthanide ions.

Vanadate also exhibited a marked tissue and species specificity in its action. Peritoneal cells of the mouse responded in a similar fashion to the rat, whereas lung and mesenteric cells of the latter species were much less reactive and human basophils and tissue mast cells of the guinea pig were essentially refractory to the anion.

The histamine releasing action of vanadate was confined to the +V oxidation state and neither vanadyl sulphate (+IV oxidation state) nor the analogous ortho-phosphate anion acted as effective secretagogues.

The precise mechanism by which vanadate induces histamine release remains to be determined. However, by analogy with previously reported effects, the anion may act to increase the intracellular concentration of calcium in the mast cell either by promoting influx or mobilization of the cation or by blocking sequestration of calcium into internal stores or extrusion into the extracellular environment. Further work will be required to resolve these possibilities.

This work was supported by grants from Fisons Pharmaceuticals and the Wellcome Trust.

Akera, T. et al (1983) Fedn Proc. 42, 2984-2988.
Jandhyala, B.S. & Hom G.J. (1983) Life Sci. 33, 1325-1340.
Pearce, F.L. (1982) Prog. med. Chem. 19, 59-109.

SK&F 93574, A HISTAMINE H_2 RECEPTOR ANTAGONIST, RELEASES HISTAMINE IN THE DOG

C.A. Jones, G. Conybeare, A. Mortenson, L.C. Catto & C.K. Atterwill, Smith Kline & French Research Ltd., The Frythe, Welwyn, Hertfordshire AL6 9AR

It was observed during pharmacological studies (unpublished observations) that some dogs treated with SK&F 93574 (a novel histamine H_2 receptor antagonist) collapsed and showed clinical signs which suggested histamine may have been released. Many other drugs are known to release histamine (see Lorenz & Doenicke, 1978). The present study was carried out to establish whether this was the case for SK&F 93574.

Three female beagle dogs each received single injections of each of SK&F 93574 (2.5 mg/kg), positive control compound polyvinyl pyrrolidone (PVP, 20 mg/kg) and sterile saline. The treatments were given at 14 day intervals by rapid intravenous infusion (through an indwelling cannula) at 0.5 ml/kg/min for 2 minutes.

Clinical observations were recorded during and after each treatment. As close a possible to 10 min after the end of the infusion a venous blood sample (nominally 20 ml) was obtained from each dog (through an indwelling cannula in a leg opposite the one used for drug administration). Pre-test blood samples were obtained during two sham experiments carried out 3 and 2 weeks prior to the first test day. The blood samples were withdrawn into heparinised syringes and then centrifuged. Aliquots of the resulting plasma were stored at -70 °C until the histamine radioimmunoassay was performed (using a kit from NMS Pharmaceuticals, Newport Beach, California, USA).

No clinical abnormalities were observed after saline treatment. On all occasions treatment with SK&F 93574 or PVP resulted in responses such as vasodilation, licking lips, head drooping, defaecation, flatulence and collapse. The severity and duration of the signs were similar for both compounds.

On the pre-test days the range of plasma histamine concentrations was from 15.2 to 18.2 ng/ml. Saline treatment resulted in concentrations of 13.2, 18.2 and 15.2 ng/ml. After PVP treatment the values measured were 300, 370 and 260 ng/ml and those after SK&F 93574 treatment were >400, 220 and 200 ng/ml.

In summary, in all three dogs intravenous infusion of SK&F 93574 produced signs typical of histamine release. These were also observed after treatment with PVP (which is known to release histamine (Ruff et al, 1967). No such changes were noted after saline treatment. The plasma histamine concentrations measured also indicated a marked histamine release after treatment with PVP or SK&F 93574, but not saline. Thus, it is concluded that intravenous infusion of SK&F 93574 into dogs is associated with histamine release. The exact mechanism of release and any association with histamine receptors on basophils or mast cells (Lichtenstein & Gillespie, 1975) awaits investigation.

Lichtenstein, L.M. & Gillespie, E. (1975) Journal of Pharmacology and Experimental Therapeutics 192, 441-450.
Lorenz, W. & Doenicke, A. (1978) Mount Sinai Journal of Medicine 45, 357-386.
Ruff, F., Saindelle, A., Dutripon, E. & Parrot, J.L. (1967) Nature 214, 279-281.

DIFFERENTIAL INOSITOL PHOSPHATE RESPONSES TO HISTAMINE, ANGIOTENSIN II AND BRADYKININ IN ADRENAL MEDULLA CELL CULTURES

R. Plevin* & M.R. Boarder, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester. LE1 7RH.

Adrenal chromaffin cells are known to be regulated by receptors to bradykinin, angiotensin II, histamine and muscarine (Livett & Marley, 1987; Forsberg et al 1986; Feldberg & Lewis, 1964). However, the mechanism by which activation of these receptors influence cellular function is not known. These receptors have been shown in a number of other tissues to stimulate the formation of the second messengers inositol-1,4,5-trisphosphate and 1,2-diacylglycerol (Berridge, 1984). Therefore we looked at phospholipid hydrolysis in chromaffin cell cultures to determine whether this could be the means by which these agonists regulate cell function.

Bovine chromaffin cells were isolated and cultured as described by Kilpatrick et al (1980). Primary cultures were prelabelled with [^{3}H]-myo-inositol (1 or 4 μCi/ml) for 32-40 hr in balanced salt solution. For pharmacological characterisation total [^{3}H]-inositol phosphate accumulated over 45 min was measured in the presence 10 mM LiCl. Extraction and separation of individual [^{3}H]-inositol phosphates was according to the method of Downes et al (1986), following incubation with agonists for 2 or 5 min.

The agonists carbachol (+ 10 μM hexamethonium), histamine, angiotensin II and bradykinin stimulated the accumulation of total [^{3}H]-inositol phosphates with EC_{50} values of 13.4 ± 4.9 μM (n = 3), 1.36 ± 0.29 μM (n = 3), 2.31 ± 0.5 nM (n = 4) and 0.488 ± 0.117 nM (n = 3) respectively. In addition responses to maximal concentrations of carbachol (+ hex), histamine and angiotensin II were fully inhibited by the corresponding antagonists atropine (10 μM), mepyramine (1 μM) and Sar^1-Thr^8-angiotensin II (30 μM) (n = 2). The B_1-agonist des-arg^9-bradykinin was virtually inactive at 1 μM indicating a B_2 receptor subtype. The maximal responses elicited by each agonist were different. Histamine was the most potent (12-fold) followed by angiotensin II (7-fold), bradykinin (5-fold) and carbachol (3-fold).

The pattern of [^{3}H]-inositol mono-, bis- and tris-phosphate formation was examined and was found to differ with respect to the agonist employed. Thus, histamine was the greatest stimulant of [^{3}H]-inositol monophosphate production at both 2 and 5 min (275 and 168%) followed by bradykinin (220 and 149%) and angiotensin II (200 and 118%). However, bradykinin was the most potent stimulant of [^{3}H]-inositol trisphosphate formation (280 and 440%) followed by angiotensin II and histamine.

Several explanations are possible for the difference in the pattern of inositol phosphate formation elicited by histamine. One possible consequence of this difference however, may be that histamine gives a relatively strong 1,2-diacyl-glycerol response while bradykinin may give a stronger IP_3 and Ca^{2+} mobilising response in cells of this type.

Berridge, M.J. (1984) Biochem. J. 220, 345.
Downes, C.P. et al (1986) Biochem. J. 238, 501.
Feldberg, W. & Lewis, G.P. (1964) J. Physiol. 171, 98.
Forsberg, E.J. et al (1986) J. Biol. Chem. 261, 4915.
Kilpatrick, D.L. et al (1980) J. Neurochem. 35, 679.
Livett, B.G. & Marley, P.D. (1987) Brit. J. Pharmac. 89, 327.

ANGIOTENSIN II AND HISTAMINE ENHANCE CYCLIC AMP ACCUMULATION IN INTACT CELLS BUT NOT ADENYLATE CYCLASE IN PERMEABILISED CELLS

M.R. Boarder*, R. Plevin & D. Marriott, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester. LE1 7RH.

Numerous reports on a variety of tissues have shown that histamine, acting on H_1 receptors, is able to enhance cyclic AMP accumulation by an indirect influence on adenylate cyclase. Angiotensin II receptors are reported to be negatively linked to adenylate cyclase and/or positively linked to phosphatidylinositol breakdown (e.g. Enjalbert et al. 1986); however, a stimulatory effect of cyclic AMP accumulation in smooth muscle cells has been reported (Nabika et al. 1985). We have looked at the effects of angiotensin and histamine on basal and prostaglandin E_1 stimulated cyclic AMP production in primary cultures of bovine adrenal medulla cells.

The cell preparation was from bovine adrenal medulla digested with collagenase, purified on Percoll and cultured as described previously (Adams & Boarder, 1987). Confluent cultures on multiwells, used after 4-7 days, consisted of chromaffin cells and contaminating cells. For experiments on cyclic AMP accumulation in intact cells, the preparation was incubated for 3 min with the drugs, followed by protein binding assay for cyclic AMP after acid extraction. For adenylate cyclase assay on permeabilised cells, cells were treated with 20 μM digitonin for 10 min, washed twice and assayed for labelled cyclic AMP formation, in the presence of [α-^{32}P]ATP, during an incubation of 15 min.

Some typical results for cyclic AMP accumulation are shown in Table I. Both angiotensin II and histamine caused a substantial increase in basal cyclic AMP; angiotensin II was powerfully synergistic with PGE_1, while histamine with PGE_1 produced a cyclic AMP increase a little above additive. The angiotensin II response was effectively inhibited by [Sar^1,Thr^8]-angiotensin II and the histamine response by mepyramine. However, there was no enhancement of basal of PGE_1 stimulated adenylate cyclase activity in permeabilised cells treated with angiotensin (Table II). Phorbol ester also enhances PGE_1 stimulated cyclic AMP accumulation in intact cells, but not adenylate cyclase in permeabilised cells.

Table I Cyclic AMP accumulation in intact cells

	Control	Angiotensin II (100 nM)	Histamine (10 μM)
No PGE_1	0.03 ± 0.002	0.39 ± 0.10	0.83 ± 0.13
PGE_1 (0.5 μM)	0.21 ± 0.04	2.53 ± 0.14	1.40 ± 0.20

Figures are pmol/well (n = 4, ± SEM)

Table II Adenylate cyclase in permeabilised cells

	Control	Angiotensin II (100 nM)	Histamine (10 μM)
No PGE_1	4415 ± 46	4066 ± 244	4691 ± 250
PGE_1 (5 μM)	6003 ± 282	6073 ± 105	6026 ± 392

cpm per well incorporated into cyclic AMP (n = 4, ± SEM)

One possible explanation for these observations is enhancement of adenylate cyclase by angiotensin II and histamine by a protein kinase C dependent mechanism; this may be lost on permeabilisation as the protein kinase diffuses into the medium.

Adams, M. & Boarder, M.R. (1987) J. Neurochem. 49, 208.
Enjalbert, A. et al. (1986) J. Biol. Chem. 261, 4071.
Nabika, T. et al. (1985) Biochem. Biophys. Res. Comm. 131, 30.

PHORBOL ESTERS BUT NOT FORSKOLIN ATTENUATE THE INHIBITORY ACTION OF ADENOSINE AT THE FROG NEUROMUSCULAR JUNCTION

A.M. Sebastião* & J.A. Ribeiro, Laboratory of Pharmacology, Gulbenkian Institute of Science, 2781-Oeiras, Portugal.

Adenosine (ADO) inhibits the output of transmitter from nerve endings. In contrast, forskolin (FSK) and phorbol esters enhance neurotransmitter release. FSK can indirectly activate protein kinase A by activating adenylate cyclase (Seamon et al., 1981), whereas phorbol esters activate protein kinase C (Castagna et al., 1982). The present work was undertaken to study the effects of ADO and 2-chloroadenosine (CADO) on neuromuscular transmission in the presence of FSK or phorbol esters (4β-phorbol, 12,13-diacetate (PDAc) and 4α-phorbol,12,13-didecanoate (PDDec)).

The experiments were carried out at room temperature (22-25°C) on the isolated nerve-sartorius muscle of the frog. The nerve was stimulated at a constant rate (0.5 Hz) with supramaximal rectangular pulses of 20 μs duration. Intracellular techniques for recording evoked endplate potentials (e.p.ps) were conventional (e.g. Ribeiro & Sebastião, 1987). The normal bathing solution (pH 7.0) contained (mM): NaCl 117, KCl 2.5, NaH_2PO_4 1, Na_2HPO_4 1, $CaCl_2$ 1.8, $MgCl_2$ 1.2. Muscle action potentials and twitches in response to nerve stimulation were prevented by increasing the Mg^{2+} concentration (9-11 mM) in the bath or by adding tubocurarine (1 μM).

FSK (0.1-2.5 μM) increased both the amplitude (6-28%) and the quantal content (6-38%) of e.p.ps without changing the membrane resting potential of the muscle fibres (n=6). In these concentrations FSK did not modify the inhibitory action of ADO (1-30 μM) on the amplitude and quantal content of e.p.ps (n=4) (see Figure 1). PDAc (100-250 nM) also increased the amplitude (16-58%) and the quantal content (21-67%) of e.p.ps without causing appreciable changes in the membrane resting potential of the muscle fibres (n=9). In the presence of PDAc (100 nM) the concentration-response curve for the inhibitory effect of ADO (1-30 μM) on e.p.ps amplitude was shifted to the right (see Figure 1) by a factor of 3.2 ± 0.3 (n=3) and that of CADO (0.3-3 μM) by a factor of 3.1 ± 0.2 (n=2). The phorbol ester, PDDec (1 μM), which is inactive on protein kinase C (Castagna et al., 1982) was devoid of effect either on the amplitude of e.p.ps or on the inhibitory action of ADO on e.p.ps amplitude (n=2).

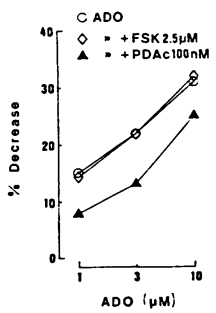

Figure 1 Comparison between the actions of FSK and PDAc on the inhibitory effect of ADO on neuromuscular transmission. The ordinates represent the percentage decreases in e.p.ps amplitude caused by ADO in the absence or in the presence of FSK (2.5 μM) or PDAc (100 nM). The results were obtained from the same endplate where FSK (2.5 μM) and PDAc (100 nM) increased e.p.ps amplitude by 27% and 26% respectively. Membrane resting potential: -95 mV. Solutions contained 10.5 mM Mg^{2+}.

These results suggest that the adenylate cyclase - protein kinase A system is not involved in the inhibitory action of adenosine on transmitter release, and that this effect of adenosine might result from interaction with protein kinase C.

Castagna, M., Takai, Y., Kaibuchi, K., Sano, K., Kikkawa, U., & Nishizuka, Y. (1982) J. biol. Chem. 257, 7847-7851.

Ribeiro, J.A. & Sebastião, A.M. (1987) J. Physiol. 384, 571-585.

Seamon, K.B., Padgett, W. & Daly, J.W. (1981) Proc. natn. Acad. Sci. U.S.A. 78, 3363-3367.

CYTOSOL TO MEMBRANE TRANSLOCATION OF CYCLIC AMP-DEPENDENT PROTEIN KINASE AS A MECHANISM OF ACTION OF β-ADRENOCEPTOR AGONISTS

Mark A. Giembycz * and Jack Diamond (introduced by Ian W. Rodger). Division of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, University of British Columbia, Vancouver, British Columbia, Canada, V6T 1W5.

There is good evidence in the literature to suggest that under physiological conditions cyclic AMP-dependent protein kinase (A-kinase) represents the sole intracellular receptor for cyclic AMP in all cell types so far studied. Although it is recognised that A-kinase is able to regulate many cellular mechanisms by phosphorylating specific cytosolic and membrane-associated protein substrates, the events which couple activation of A-kinase to the expression of the observed physiological response are poorly understood. This abstract describes the results obtained from studies designed to test the hypothesis formulated by Krall et al (1978), that translocation of A-kinase from the cytosol to membrane-located substrates is part of the biochemical cascade by which cyclic AMP-elevating drugs exert their biological effects in many cell types.

Soluble and particulate A-kinase from vehicle- and drug-treated guinea-pig lung was prepared using well established homogenisation, centrifugation and detergent-solubilisation techniques. Guinea-pig lung was used for these studies since it is one of the richest sources of A-kinase known. The inactive holoenzymes (Type I and Type II) and free catalytic subunits were separated by anion-exchange chromatography over diethylaminoethyl-cellulose and assayed for A-kinase activity using a modification of the method initially documented by Cook et al (1982) with either mixed histones or the synthetic heptapeptide, kemptide, as the substrate.

The results, presented in Table 1, illustrate that isoprenaline produced a concentration-dependent activation of A-kinase in guinea-pig lung that was associated with concentration-dependent translocation of A-kinase from the soluble to the particulate fraction. When isoprenaline was used in combination with forskolin almost complete activation and translocation of the enzyme was observed.

Table 1. Effect of Isoprenaline on the Distribution of A-kinase in Guinea-pig Lung (n = 4; a = $P < 0.05$).

	Free Catalytic Subunits (% Total Enzyme)		Inactive Holoenzymes (Type I + II) (% Total Enzyme)		% Original Enzyme in Fraction	
Treatment	Soluble	Particulate	Soluble	Particulate	Soluble	Particulate
Control	3.8 ± 0.4	4.6 ± 0.9	91.2 ± 9.3	95.7 ± 11.7	100	100
Isoprenaline (50 nM)	3.8 ± 0.3	6.2 ± 1.0	97.9 ± 11.1	96.0 ± 7.6	102.0 ± 10.7	102.6 ± 4.3
Isoprenaline (500 nM	4.1 ± 0.4	47.0 ± 8.3[a]	80.0 ± 4.6	91.4 ± 9.2	84.1 ± 6.5	138.5 ± 13.7[a]
Isoprenaline (5 μM)	4.9 ± 0.4	67.0 ± 15.6[a]	64.9 ± 3.9[a]	74.3 ± 15.3[a]	68.8 ± 13.2[a]	141.3 ± 24.8[a]
Isoprenaline (50 μM)	5.6 ± 0.3[a]	100.3 ± 36.8[a]	33.6 ± 4.6[a]	78.0 ± 6.4[a]	39.2 ± 5.9[a]	179.7 ± 47.1[a]
Isoprenaline (10 μM) + Forskolin (10 μM)	8.1 ± 0.5[a]	136.4 ± 33.7[a]	0.8 ± 0.1[a]	46.1 ± 12.1[a]	8.9 ± 1.0[a]	182.5 ± 27.7[a]

The data presented herein suggest that activation of A-kinase followed by translocation of the free catalytic subunits from the cytosol to membrane-associated protein substrates may represent a physiologically important mechanism intimately involved with the regulation of β-adrenoceptor-mediated phenomena. If smooth muscle relaxation is considered then this hypothesis is strengthened since the enzyme systems that have been proposed to ultimately effect relaxation are, almost exclusively, integral components of the plasmalemma and/or endoplasmic reticulum.

Krall, J.F., et al. (1978). Arch.Biochem.Biophys., 187, 1-11.
Cook, P.F., et al. (1982). Biochemistry., 21, 5794-5799.

INVESTIGATION INTO THE RELATIONSHIP BETWEEN INTRACELLULAR CALCIUM CONCENTRATION ($[Ca^{2+}]_i$) AND AGGREGATION IN PLATELETS

A.L. Lees, C.H. Orchard[1] and M.A. Orchard*, University Department of Medicine and [1]Department of Physiology, The General Infirmary, Leeds, LS1 3EX.

Cyclic AMP (cAMP) and Ca^{2+} are intracellular mediators with opposing actions in platelets. We have investigated the effect of increasing cAMP on the relationship between $[Ca^{2+}]_i$ and aggregation in collagen and thrombin stimulated platelets. Cyclic AMP was increased either by inhibition of phosphodiesterase III with SK&F 94120 (Gristwood et al, 1985) or by stimulation of adenylate cyclase with PGI_2.

Platelets were prepared from citrated venous plasma and loaded with aequorin using a method modified from that reported by Vickers and Mustard (1986). Briefly, platelets were washed with HEPES buffered solution containing 10 mM EGTA and 1 uM PGE_1 before being incubated at room temperature in a hypotonic buffer containing 5 uM ATP and 100 ug ml^{-1} aequorin for 15 min followed by 15 min in isotonic buffer containing 2 mM $MgCl_2$. These aequorin loaded platelets were then washed in a Hepes/Tyrodes/albumin buffer (HTA) containing 0.9 uM PGI_2 before being suspended in HTA. The extracellular $[Ca^{2+}]$ was then restored to 1 mM, the platelet count adjusted to 2 x 10^8 ml^{-1} and aggregation and aequorin luminescence recorded using a modified Platelet Ionised Calcium Aggregometer (Chronolog, U.S.A.). Platelet $[Ca^{2+}]_i$ was calculated by the method described by Allen and Blinks (1978) and significance was assessed using the paired 't-test'.

Sub-maximal concentrations of collagen (10ug ml^{-1}) and thrombin (0.05U ml^{-1}) were used which induced comparable increases in $[Ca^{2+}]_i$ and aggregation. SK&F 94120 (20 uM for 3 min) significantly decreased the collagen induced aggregation from 49.5 $\pm$ 2.2% to 19.5 $\pm$ 5.0% ($p < 0.01$) and the change in $[Ca^{2+}]_i$ from 1230 $\pm$ 86 nM to 613 $\pm$ 90 nM ($p < 0.01$, $n = 6$). However thrombin induced aggregation was not inhibited even though $[Ca^{2+}]_i$ was decreased significantly from 1246 $\pm$ 115 nM to 754 $\pm$ 61 nM ($p < 0.02$, $n = 5$). The effects of raised cAMP on thrombin induced aggregation and $[Ca^{2+}]_i$ were investigated further. Inhibition of aggregation only followed inhibition of $[Ca^{2+}]_i$ when either lower doses of thrombin or a stronger stimulus for raising cAMP was used. Thus thrombin (0.05 U ml^{-1}) induced aggregation was reduced from 43.7 $\pm$ 2.1% to 5.1 $\pm$ 4.0% ($p < 0.001$) by 10 nm PGI_2 and change in $[Ca^{2+}]_i$ from 1162 $\pm$ 263 nM to 133 $\pm$ 97 nM ($p < 0.05$, n=4).

The above results confirm previous observations that raised cAMP levels inhibit intracellular calcium mobilization (Lanza et al. 1987). In addition they suggest that inhibition of $[Ca^{2+}]_i$ is not the sole determinant for inhibition of thrombin induced aggregation.

Allen, D.G. & Blinks, J.R. (1978) Nature 273, 509.
Gristwood, R.W. et al. (1985) Br. J. Pharmacol. 85, 224P.
Lanza, F. et al. (1987) Thromb. Res. 45, 477.
Vickers, J.D. & Mustard, J.F. (1986) Biochem. J. 238, .