ACUTE TOLERANCE OF SOMATO-CARDIOVASCULAR REFLEXES TO FENTANYL: EFFECT OF NOCICEPTIVE STIMULATION

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Acute tolerance to the analgesic effects of fentanyl in rats occurs within four days (Colpaert et al, 1980). The effect of pain on tolerance to opiates is not agreed (Ferguson and Mitchell, 1969, Colpaert et al, 1980). The present study investigated the rate of development of acute tolerance to the effects of the intravenous administration of fentanyl on the cardiovascular reflexes mediated by group III and IV, cutaneous nerve fibres (Whitwam et al, 1979), and its interaction with such nociceptive stimulation.

Experiments were performed on three groups of anaesthetised (α-chloralose) paralysed (suxamethonium) and artificially ventilated dogs. Group A received a bolus dose of 100μg kg^{-1} of fentanyl. Group B, before receiving the same dose, was conditioned for 3 hours with 10 increasing doses of fentanyl to produce a logarithmic increase of the plasma concentration, equivalent to a bolus of 100μg kg^{-1}. Each dose of fentanyl was followed by supramaximal electrical stimulation of the exposed desheathed radial nerve. Group C was treated in the same way as group B but without nerve stimulation during the conditioning period.

In group A, 100μg kg^{-1} of fentanyl caused a highly significant reduction of the evoked responses to electrical stimulation in arterial pressure and heart rate. In group B the cardiovascular responses after the bolus dose of 100μg kg^{-1} were significantly reduced by 19% and 15% at 5 and 10 min. respectively compared to those at the end of the conditioning period ($p < 0.0008$ and 0.025 respectively), but had returned to those values by 15 min. The fentanyl plasma concentrations, measured by radio-immunoassay, were two to three times higher in group B than in Group A following the 100μg kg^{-1} dose of drug. In Group C, following the same drug conditioning but without stimulation there was no effect on the evoked cardiovascular responses by 100μg kg^{-1} of fentanyl. Recovery times to pre-fentanyl controls, following the 100μg kg^{-1} bolus dose were 90 and 50 min. for groups A and B respectively.

During the conditioning period in the group where nociceptive stimuli were applied, initial small doses of fentanyl were ineffective in depressing the somato-cardiovascular responses. As the doses were increased a progressive effect of fentanyl was seen up to the development of tolerance, which occurred by 2½ hr., after which increasing doses of the drug had no further effect. Moreover the reflex depression persisted unchanged until the drug was withdrawn. In the unstimulated group tolerance occurred before any analgesic effect could be demonstrated. This study shows that nociceptive stimulation, when combined with drug administration, delayed the development of complete tolerance to fentanyl.

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Colpaert, F.C. et al (1980). J.Pharmacol.Exp.Ther., 213, 418-424.
Ferguson, R.K., and Mitchell,C.L. (1969). Clin.Pharmacol.and Ther.,10,372-382.
Whitwam, J.G. et al (1979). Brain Research. 165, 219-233.

A COMPARISON OF THE CARDIOVASCULAR EFFECTS OF MEPTAZINOL AND NALOXONE FOLLOWING ANAPHYLACTIC SHOCK IN ANAESTHETIZED RATS

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Meptazinol, an analgesic with opiate antagonist activity (Goode & White, 1971) reversed endotoxic (Paciorek & Todd, 1981) and hypovolaemic (Chance, Todd and Waterfall, 1981) shock. We have investigated the cardiovascular actions of meptazinol administered both before and after induction of systemic anaphylactic shock.

Female normotensive rats were immunised with 1ml of $10\mu g.ml^{-1}$ ovalbumin in aluminium hydroxide gel, 0.5ml into each hind limb (i.m.) and with B.pertussis 1×10^{10} killed organisms as adjuvant (i.p.). Eight days later rats were anaesthetised (i.p.) with a mixture of urethane ($800\ mg.kg^{-1}$) and chloralose ($60mg.kg^{-1}$). Blood pressure and heart rate were monitored from a cannula inserted into the left common carotid artery, and drugs were administered i.v. via the left jugular vein, unless otherwise stated. In all cases mepyramine maleate ($0.1mg.kg^{-1}$ s.c.) was administered 10 min prior to the induction of shock. Systemic anaphylactic shock was produced by slow i.v. administration of antigen ($0.05mg.kg^{-1}$). Meptazinol ($17mg.kg^{-1}$ i.m.), naloxone ($10mg.kg^{-1}$) and saline vehicle ($1ml.kg^{-1}$) were administered 5-15 min before production of shock.

In the saline vehicle pretreated group, M.A.P. fell by 62mmHg and heart rate by 52 $bt.min^{-1}$ over the 30 min observation period following anaphylactic shock.

Meptazinol ($17mg.kg^{-1}$ i.m.) 15 min prior to challenge, completely inhibited the hypotension and bradycardia evoked by the antigen ($p < 0.001$).

Naloxone ($10mg.kg^{-1}$) 5 min prior to challenge, partially inhibited the fall in M.A.P. by 26mmHg ($p < 0.001$) and had no consistent effect upon heart rate over the 30 min observation period. The effects of meptazinol on M.A.P. and heart rate were significantly greater ($p < 0.001$) than those of naloxone.

Meptazinol (2, 6 and 17 $mg.kg^{-1}$ i.m.), naloxone ($10mg.kg^{-1}$) and saline vehicle ($1ml.kg^{-1}$) were administerd 30 min after antigen and M.A.P. and heart rate monitored for a further 30 min to investigate effects produced by drugs administered after anaphylactic shock.

Meptazinol (6 and 17 $mg.kg^{-1}$ i.m.) significantly increased M.A.P. by 39mmHg ($p < 0.001$) above control values. Heart rate rose by 55-65 $bt.min^{-1}$ over the 30 min observation period, but was not significantly different to control values. Meptazinol at a lower dose, $2mg.kg^{-1}$ i.m., raised M.A.P. by 13mmHg ($p < 0.05$) and had no consistent effect upon heart rate.

Naloxone ($10mg.kg^{-1}$) increased M.A.P. by 17mmHg ($p < 0.001$) and had no consistent effects upon heart rate over the 30 min observation period. Saline vehicle $1ml.kg^{-1}$ had no significant effect upon M.A.P. and heart rate.

Unlike naloxone, meptazinol ($17mg.kg^{-1}$ i.m.) predose completely inhibited the fall in M.A.P. induced by antigen challenge. These data further provide evidence for the use of meptazinol in shock states characterised by low blood pressure.

We are grateful to Endo Laboratories for supplying naloxone hydrochloride.

Chance, E. et al (1981) Br. J. Pharmac., 74, 930 P.
Goode, P.G. & White, A.C. (1971) Br. J. Pharmac. 43, 462-463P.
Paciorek, P.M. and Todd, M.H. (1981) Br. Pharmac. Soc. London meeting.

HYPOTENSIVE ACTIONS OF β_2 AGONISTS IN THE GANGLION-BLOCKED ANGIOTENSIN II-SUPPORTED RAT

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Stimulation of vascular ß adrenergic receptors (β_2 subtype, Lands et al., 1967) results in vasodilation. However, in vivo studies on vasodilators are frequently hampered since the vasodilation-induced reduction in blood pressure (BP) causes reflex tachycardia and secondary vasoconstriction. This may be overcome by using pithed or ganglion-blocked animals in which the low BP can be reelevated by infusion of a pressor substance. Such a model, the ganglion-blocked angiotensin II-supported rat, has recently been described (Deitchman et al., 1980) and has been employed here to investigate the vasodilatory action of some ß agonists.

Anaesthetized male Wistar rats (360-400g), cannulated for BP recording (carotid artery) and i.v. drug administration (jugular and penile vein), were i.v. injected with chlorisondamine (2.5mg/kg) and phenoxybenzamine (5mg/kg). 25 min later they were perfused with Ag II (1.5µg/kg/min at 20µl/min) through the jugular vein. This dose of Ag II produced the same average (integrated) BP as before ganglion blockade (118±2mmHg before ; 117±4mmHg after, n=22). Dose-response curves were constructed for ß agonists by injecting increasing concentrations every 10 min. ID_{20} (20% reduction of BP) were calculated from logit/log plots of the data giving : procaterol, 0.45±0.08 ; isoprenaline, 3.1±0.08 ; salbutamol, 12.4±0.8 ; adrenaline, 81.1±4.1 ; noradrenaline 666.8±159.3 and isoxsuprine 889.4±80.6µg/kg, n=5-9). Since the doses of procaterol employed did not increase heart rate in phenoxybenzamine-treated ganglion-blocked rats it was considered to exert a pure β_2 and was therefore used to examine the action of ß-blocking drugs which were infused together with the Ag II. Atenolol, IPS 339 and propranolol (1µg/kg/min, n=4-6) produced respectively 1.3, 9.1 and 15 fold parallel inhibitions of the procaterol curve, whilst butoxamine (20µg/kg/min, n=6) produced a 6.6 fold inhibition. Maximum reduction of BP (49.1±1.5% alone, n=21 ; 49.2±0.8% with ß-blockers, n=21) was not affected.

Ganglion blockade and phenoxybenzamine treatment of SHR (BP 177±3mmHg, n=20) and WKY (BP 109±3mmHg, n=18) rats produced disproportionate reductions in BP (SHR, -87±3mmHg ; WKY, -36±3mmHG, $p < 0.001$) but Ag II infusion induced the same increases in BP (SHR, +54±3mmHg ; WKY,+57±3mmHg). Administration of procaterol (0.4µg/kg) to these rats produced a hypotension which was significantly greater ($p < 0.001$) in SHR (881±78mmHg.min, n=5) than in WKY (424±26mmHg.min, n=6) rats over 30 min. There was no difference in the response of WKY and Wistar (476±52mmHg.min, n=6) rats. Similarly, adrenaline (60µg/kg) induced a greater hypotensive effect in SHR than in WKY rats over 20 min (SHR, 746±86mmHg.min, n=6 ; WKY, 500±35mmHg.min, n=6 ; $p < 0.02$).

The results confirm the potent hypotensive action of β_2 agonists and suggests that the model employed appears suitable for investigating the vascular effects of β_2 agonists and antagonists. The results obtained with SHR indicate that their hypertension may be partly maintained by an increased sympathetic output, however, the mechanism behind their increased sensitivity to β_2 stimulation is unknown.

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Lands, A.M. et al. (1967) Nature, 214, 597-598
Deitchman, D. et al. (1980) J. Pharmac. Methods, 3, 311-321

EVIDENCE OF INDIRECT ACTION OF HISTAMINE AND PEA IN GUINEA-PIG AND RAT ATRIA

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Low concentrations of histamine are known to increase heart rate and force of contraction. This action may result from direct activation of histamine receptors (Trendelenburg, 1960) or, indirectly via catecholamine release, (Laher & McNeill, 1980a). Steinberg & Holland (1975) suggest that both mechanisms may exist. The H_1-receptor agonist, 2-pyridylethylamine, (PEA) has been reported to release catecholamines in guinea pig heart (Broadley & Wilson, 1978; Laher & McNeill, 1980b). This work also suggests that both histamine and PEA have a dual mode of action.

Separated right and left atria from rats and guinea pigs were suspended in Locke solution at 32°C, or in Locke solution containing sufficient KCl (15 mM) to cause partial depolarisation: this procedure makes right atria quiescent and left atria unresponsive to electrical pacing.

In guinea pig right atria, propranolol (4×10^{-7}M) partially antagonised the positive chronotropic and inotropic effects of histamine (1×10^{-7} - 1×10^{-4}M) suggesting that the responses to histamine are mediated in part, via catecholamine release. In rat right atria, high doses of histamine (5×10^{-3}M) cause negative chronotropic effects which are not affected by atropine (1×10^{-6}M) indicating that the slowing is not related to acetylcholine release as suggested by Laher & McNeill (1980a).

In rat depolarised right atria, both histamine and PEA restored contractile activity, which was abolished by mepyramine (1×10^{-6}M) or propranolol (4×10^{-7}M). This suggests that both agonists cause release of catecholamines by an action on the H_1-receptors present in this tissue (Abdullah et al, 1981).

In guinea pig left driven atria, the positive inotropic effect of histamine (1×10^{-5}M) was partially blocked by propranolol (4×10^{-7}M) lending support to the suggestion of catecholamine release.

In guinea pig left, depolarised atria, both PEA and histamine restored the responses to electrical pacing, and these were partially blocked by propranolol (4×10^{-7}M). At 1×10^{-6}M propranolol the PEA-restored contractions were completely abolished, but this may reflect a non-specific action of this high dose.

In conclusion, these results suggest that in guinea pig and rat atria, both histamine and PEA have actions mediated directly via histamine receptors, and indirectly via the release of catecholamines.

Abdullah, F.M. et al (1981) Br.J.Pharmac. 74, 937P
Broadley, K.J. & Wilson, C. (1978) Br.J.Pharmac. 64, 387P
Laher, I. & McNeill, J.H. (1980a) Can.J.Physiol.Pharmac. 58, 1114
Laher, I. & McNeill, J.H. (1980b) Proc.West.Pharmac.Soc. 23, 41
Steinberg, M.I. & Holland, D.R. (1975) Eur.J.Pharmac. 34, 95
Trendelenburg, U. (1960) J.Pharmac.exp.Ther. 130, 450

CARDIAC EFFECTS OF M66 (1-4(-AMINOPHENYL)-6,7-DIMETHOXY-3,4-DIHYDRO-ISOQUINOLINE)

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One of a series of compounds structurally related to papaverine, M66 (1-(4-aminophenyl)-6,7-dimethoxy-3,4-dihydro isoquinoline) has been selected for further investigation due to its cardioselectivity and positive inotropic and negative chronotropic actions (Anderson, Foulkes, Goadby, Hooper, Thonoor, 1981).

Isolated guinea-pig left atria were mounted in McEwens solution at 37°C and stimulated electrically at 1.5Hz with 1ms duration. Force of beating was measured isometrically by a transducer (Grass FT03) and displayed on a Devices M2 recorder. Cumulative addition M66 (3×10^{-6} - 10^{-4}M) produced concentration-dependent increases in contractile force up to a maximum of 103 ± 23%. Under the same conditions, papaverine and ouabain produced bell-shaped concentration-response curves with maximum increases in force of 45 ± 14% and 106 ± 13% respectively.

Cumulative addition of M66 (3×10^{-6} - 10^{-4} M) to isolated spontaneously beating paired guinea-pig atria gave an increase in contractile force of 108 ± 14%. A concurrent concentration-dependent decrease in heart rate, up to -58 ± 7% at 10^{-4}M, was also seen. Pretreatment with propranolol (200 ng/ml) or reserpine (5 mg/kg i.p.) made no significant difference ($p < 0.05$) to the force or rate responses obtained with M66. Under the same conditions dobutamine (3×10^{-8} - 3×10^{-6}M) produced larger increases in force but these were accompanied by increases in rate of beating. Both effects of dobutamine were abolished by pretreatment with propranolol or resperine.

The effect of M66 upon the effective refractory period (ERP) of the isolated guinea-pig left atrium was measured by the method of French and Scott (1979). M66 (5×10^{-5}M) increased the ERP from a control of 121 ± 3 ms to 150 ± 4 ms which is comparable to the increase obtained with quinidine at a concentration of 10^{-4}M.

Rats (300-400g) anaesthetised with 75mg/kg pentobarbitone were used to measure changes in blood pressure and heart rate at infusion rates of 2 to 39 µmoles/hr M66. Heart rate was reduced throughout the range of dosage and at infusion rates above 11 µmoles/hr M66 caused decreases in arterial blood pressure.

In urethane (1.5 g/kg) anaesthetised guinea-pigs (800-1000g) M66 infused at a rate of 25 µg/min decreased heart rate from 254 ± 9 to 209 ± 11 b.p.m. ($p < 0.01$, n=4) after 30 minutes infusion. At this infusion rate there were no significant changes in QRS complexes, P-waves, P-Q or R-T intervals of the ECG records although there was an increase in the voltage of the T-wave. Following M66 infusion subsequent ouabain infusion (10 µg/kg/min) induced arrhythmia after 176 ± 12 µg/kg ouabain compared to an unpretreated control value of 223 ± 0.1 ug/kg ouabain.

Thus M66 shows evidence of positive inotropism in vitro and negative chronotropic effects in vivo and in vitro. The mechanism of action of M66 does not involve catecholamines and the pattern of activity shows several differences from that of ouabain.

Anderson, A.A., et al (1981). Br. J. Pharmac., 74, 938P.
French, A.M. & Scott, N.C. (1976). Br. J. Pharmac., 66, 19-24.

THE CENTRAL α_1- AND α_2-ADRENOCEPTOR INVOLVEMENT IN THE CARDIOVASCULAR ACTION OF UK14,304 IN THE CONSCIOUS CAT

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We recently showed that the hypotensive action of clonidine was susceptible to both α_1- and α_2- adrenoceptor blockade in the conscious renal hypertensive cat (Beckett & Finch 1981a). We have now studied the central effects of a more selective and more potent α_2- adrenoceptor agonist, UK14,304 (Beckett & Finch 1981b, Cambridge 1981).

The selective α_1- adrenoceptor antagonist prazosin, yohimbine and its stereoisomers corynanthine and rauwolscine (Weitzell et al 1979), and the selective α_2-adrenoceptor antagonist RS21361 (Michel & Whiting 1981) were given intracerebroventricularly (i.c.v.) 30 minutes prior to UK14,304.

UK14,304 (12.5 µg i.c.v.) caused hypotension and bradycardia which was maximal 60-90 minutes after administration. Sedation accompanied these effects. 100 µg of prazosin, corynanthine, yohimbine and rauwolscine, and 800 µg of RS21361 significantly antagonised the hypotensive action of UK14,304 but not the bradycardia (Table 1). Yohimbine, rauwolscine and RS21361 also appeared to reduce the sedation induced by UK14,304. This profile of the antagonism of the hypotensive action of UK14,304 closely resembles that obtained with clonidine. The blockade by prazosin and the lack of differential antagonism by yohimbine and its isomers would suggest UK14,304 to act via α_1- adrenoceptors. However, UK14,304 is selective for α_2- adrenoceptors and RS21361, a highly selective α_2- adrenoceptor antagonist, significantly blocked UK14,304.

These results may imply that the hypotensive effect of clonidine-like drugs is mediated by both α_1- and α_2- adrenoceptors or, possibly, that these central α-adrenoceptors have pharmacological differences to peripheral α_1- and α_2- subtypes. In contrast, the α- adrenoceptors mediating sedation appeared to be of the α_2-subtype.

Table 1 - The interaction of UK14,304 with α- adrenoceptor antagonists in the conscious cat.

Dose given i.c.v.	Max % Δ from resting levels B.P. (Mean ± S.E. mean)	H.R. (Mean ± S.E. mean)
UK14,304 - 12.5 µg (alone)	-36.5 ± 2.1	-30.4 ± 6.9
+ prazosin - 100 µg	-12.2 ± 4.0**	-23.3 ± 4.7
+ corynanthine - 100 µg	-21.5 ± 1.6**	-32.6 ± 2.8
+ yohimbine - 100 µg	-20.1 ± 5.7*	-27.9 ± 3.5
+ rauwolscine - 100 µg	-21.5 ± 2.5**	-26.8 ± 3.0
+ RS21361 - 800 µg	-18.7 ± 6.2*	-34.8 ± 5.2

n = 5-7 for all groups

* Significantly different from controls ($P < 0.05$) unpaired students t-test

** Significantly different from controls ($P < 0.01$) unpaired students t-test

M.A.P. and H.R. = 128 ± 6.2 mmHg and 182 ± 8.7 beats/min respectively (Mean ± S.E. mean n = 20).

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Beckett, P.J. & Finch, L. (1981a). Br. J. Pharmac., 74, 190.
Beckett, P.J. & Finch, L. (1981b). Br. J. Pharmac., (this meeting)
Cambridge, D. (1981). Eur. J. Pharmac., 72, 413-416.
Michel, A.D. & Whiting, R.L. (1981). Br. J. Pharmac., 74, 255-256P.

THE EFFECTS OF ATROPINE AND β-ADRENOCEPTOR ANTAGONISTS ON EXERCISE-INDUCED TACHYCARDIA IN THE CAT

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A method of recording exercise tachycardia in cats has been developed. Blood pressure was recorded directly by the method of Burden, Blaber and Natoff (1979) with the catheter tip in the descending aorta.

Single animals were placed in a metal drum 25 cm deep and 110 cm diameter mounted horizontally onto a central spindle. The front of the drum was closed by placing a fixed piece of perspex as close as possible whilst still allowing free rotation.

The indwelling catheter was connected to a Statham P23 Db blood pressure transducer by polythene tubing filled with heparinised saline. Heart rate was recorded by a tachometer triggered from the blood pressure pulse. Consistent maximal increases in heart rate were produced by revolving the drum by hand at 20 RPM for thirty seconds; therefore this rate was used and also 2 RPM for thirty seconds which produced a sub-maximal increase in heart rate. The cats were trained in the apparatus before experiments were performed.

2 RPM produced a tachycardia of approximately 55 beats/min and 20 RPM, approximately 120 beats/min. In a cross-over design experiment using 2 groups of 5 animals, placebo treatment produced no significant differences from control.

Bufuralol (10 mg/kg po) a non-selective β-adrenoceptor antagonist reduced the 20 RPM response for at least 4 hours but not the 2 RPM response. A combination of bufuralol (10 mg/kg po) and atropine sulphate (1 mg/kg po) reduced both 2 RPM and 20 RPM responses; the 20 RPM response being reduced greater than by bufuralol alone. Bufuralol alone or with combined therapy had no effect on resting heart rate; atropine alone produced tachycardia.

We conclude that, similar to man, moderate exercise produces tachycardia by vagal withdrawal and severe exercise by sympathetic stimulation (Robinson et al, 1966; Martin et al, 1974). A comparison between propranolol (10 mg/kg po) and bufuralol (10 mg/kg po) on the 20 RPM response showed propranolol to have a duration of action of 6 hours and bufuralol, 12 hours. When isoprenaline tachycardia was used propranolol reduced the response for 36 hours and bufuralol for 48 hours.

These studies have shown that exercise tachycardia in the cat is a useful guide to activity of β-adrenoceptor antagonists in man.

Burden, D.T., Blaber, L.C. and Natoff, I.L. (1979) Pharmacol. Ther. 5, 99-102.

Martin, C.E., Shaver, J.A., Leon, D.F., Thompson, M.E., Reddy, P.S. and Leonard, J.J. (1974) J. Clin. Invest. 54, 104-115.

Robinson, B.F., Epstein, S.E., Beiser, G.D. and Braunwald, E. (1966) Circ. Res. 19, 400-411.

THE CARDIAC STIMULANT EFFECTS OF ISOPRENALINE IN THE CONSCIOUS DOG: STUDIES ON THE ROLE OF β_1 AND β_2 ADRENOCEPTORS

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The myocardial effects of isoprenaline in vivo may result from reflex activity in response to β_2 mediated vasodilatation as well as the more commonly accepted direct β_1 stimulation. To determine the parts played by each of these mechanisms, isoprenaline was infused into conscious dogs before and after the β_1 antagonist practolol (Dunlop & Shanks, 1968) or the β_2 antagonist ICI 118551 (Bilski et al, 1980).

Four conscious male beagle dogs were used. Lead II ECG was recorded and the arterial pulse detected by a strain gauge placed on an exteriorised carotid loop. Inotropic changes were assessed by measurement of the time interval between the Q wave of the ECG and the arrival of the arterial pulse at the carotid loop. This QA interval has been shown within our own laboratories and by others (Jackson, 1974) to be a non-invasive measure of cardiac contractile state which is largely independent of changes in BP or heart rate (HR). Isoprenaline (Suscardia, Pharmax) was administered by stepwise i.v. infusion, doubling the rate of infusion every 15 min. QA interval and HR were determined at control and for each infusion rate. On separate occasions these infusions were repeated 10 min after 1 mg/kg practolol hydrochloride or 0.2 mg/kg ICI 118551 given i.v. and the values of QA interval and HR re-determined. Values in response to these infusions were compared to those obtained previously in the absence of blockade.

Isoprenaline produced dose dependent shortening of QA interval (positive inotropism) and increases in HR. The former was partially inhibited by β_1 or β_2 blockade whilst the tachycardia was abolished by β_2 but not β_1 blockade (see Figure I).

In the conscious dog therefore β_2 adrenoceptor stimulation plays a major role in the chronotropic action of isoprenaline, presumably via vagal withdrawal resulting from β_2 arteriolar vasodilatation. Reflex sympathetic activity may contribute to the inotropic response to isoprenaline but direct effects on cardiac β_1 and β_2 adrenoceptors cannot be excluded.

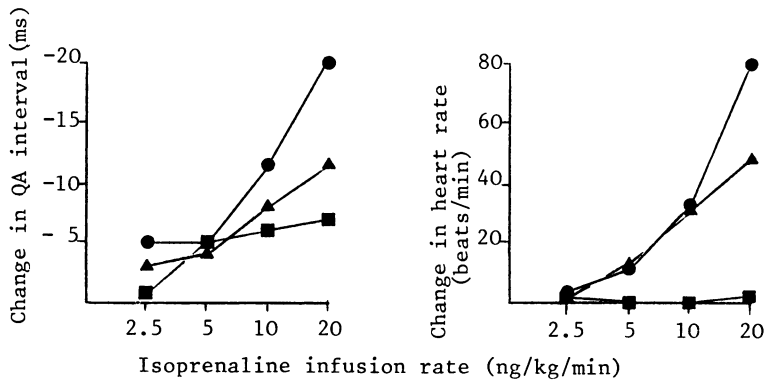

Figure 1. Inotropic and chronotropic responses to stepwise i.v. infusion of isoprenaline before (●—●) and after selective β_1 (▲—▲) or β_2 (■—■) blockade.

We wish to thank Mr. M. Douthwaite for his invaluable technical assistance.

Bilski, A. et al (1980) Br.J.Pharmac. 69, 292P
Dunlop, D. & Shanks, R.G. (1968) Br.J.Pharmac. 32, 201-218
Jackson, D.M. (1974) Lancet II, 1457

STRUCTURE ACTIVITY RELATIONSHIPS WITH REGARD TO ANTIARRHYTHMIC ACTIVITY OF STEROIDAL VICINAL 2,3-AMINO ALCOHOLS

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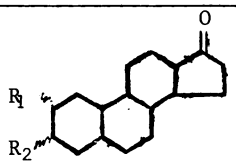
3α-amino-2β-hydroxy-5α-androstan-17-one (ORG 6001) exerts antiarrhythmic actions (Vargaftig et al, 1975). The present study evaluates the relative activities of 3-amino-2-hydroxy and 2-amino-3-hydroxy isomers (Campbell et al, 1979) of ORG 6001 on 1) the action potential characteristics of sheep Purkinje fibres 2) the maximum following frequency of isolated driven guinea-pig left atria (MFF) and 3) antagonism of aconitine induced arrhythmias in mice.

Table 1 Activities of isomers of ORG 6001 *p<0.001 ( ) 95% confidence limits



	R_1	R_2	Mean % changes in action potential characteristics of Purkinje fibres at drug concentrations of 8μg/ml (†preliminary result only) MRD	APD_{50}	APD_{90}	Mouse Aconitine ED_{50} (mg/kg)	Guinea-Pig atria. ED_{25} (μg/ml) for reduction in MFF
6001	βOH	αNH_2	-34*	-31*	-19*	15(8-18)	93(58-269)
I	βOH	$\alpha NHCH_3$	-46*	-44*	-29*	14(6-22)	84(47-586)
II	βOH	βNH_2	-82*	-3	+22*	30(20-53)	48(31-113)
III	βOH	$\beta NHCH_3$	†-63	†+8	†+33	13(10-21)	25(14-182)
IV	αOH	αNH_2	-21*	-22*	-2	INACTIVE	INACTIVE
V	βNH_2	αOH	-9	-31*	-14*	INACTIVE	INACTIVE
VI	$\beta NHCH_3$	αOH	-11	-28*	-11*	INACTIVE	INACTIVE
VII	βNH_2	βOH	-20*	-7	+12*	58(36-68)	53(37-102)
VIII	$\beta NHCH_3$	βOH	-49*	-21*	+28*	30(20-39)	50(34-143)
IX	αNH_2	αOH	-28*	-46*	-21*	31(NS)	INACTIVE
X	$\alpha NHCH_3$	αOH	-28*	-26*	-3	28(15-38)	102(47-859)

Action potential duration (APD) was more influenced by the bond angles than by the nature of the substituent e.g. compounds with 2β,3α-substituents shortened both APD_{50} and APD_{90} (6001, I, V and VI). Replacing the 3α-substituents of these compounds with a β-substituent resulted in prolongation of APD_{90} and abolition of APD_{50} shortening except in the case of VIII (APD_{50} shortened). In addition the effects to reduce the maximum rate of depolarisation (MRD) tended to increase with 3β-substitution. Effects on MRD were also influenced by the nature of the substituent; e.g. retaining the bond angles of 6001 and I, but reversing the amino and alcohol substituents reduced the effects on MRD (V and VI).

The order of potency in reducing MFF roughly paralleled the reduction of MRD although changes in APD may have influenced the results e.g. VII (which prolonged APD_{90}) was more potent and IX (which markedly shortened APD_{50}) was less potent than predicted from effects on MRD. Compounds having relatively little effect on MRD also showed little or no activity in the aconitine test.

In conclusion, a reduction in MRD but not in APD was associated with antiarrhythmic activity. Overall, 2β-hydroxy,3β-amino substitution yielded the most active compounds (II and III) whereas 2β-amino,3α-hydroxy compounds were the least active (V and VI).

Campbell, M.M. et al (1979) J.Chem.Soc. 1, 2235
Vargaftig, B.B. et al (1975) J.Pharm.Pharmac. 27, 697

COMPARISON OF THE VASODILATOR EFFECTS OF AMRINONE AND 3-ISOBUTYL-1-METHYL-XANTHINE (IBMX)

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Amrinone is a new cardiotonic agent that has been shown to possess both positive inotropic and vasodilator activity (Alousi et al. 1979; Meisheri et al. 1980). To date, however, its mechanisms of action are unknown. We report here the results of an investigation in which the effects of amrinone and IBMX have been compared on tension responses and cyclic nucleotide levels in vascular smooth muscle.

Pulmonary artery strips taken from NZW rabbits were suspended in Krebs-Henseleit solution at 37°C and gassed with 5% CO_2 in O_2. After equilibration the preparations were constricted near maximally with phenylephrine (1×10^{-5}M).

Concentration-dependent relaxations of the constricted arteries were induced by amrinone (1×10^{-5}M to 1×10^{-3}M) and IBMX (5×10^{-7}M to 1×10^{-4}M). Cyclic nucleotide levels were measured at different levels of drug-induced relaxation (10%, 25%, 50%, 75% and 100%). The results are summarised in Table 1.

Table 1 Effect of amrinone and IBMX on tension and cyclic nucleotide levels in phenylephrine (1×10^{-5}M) constricted pulmonary arteries. The values are mean ± s.e. mean of at least 5 preparations.

Drug Concentration	% Relaxation	cAMP (pmol/mg)	cGMP (pmol/mg)
Control (Phenylephrine 10^{-5}M)		0.23 ± 0.01	0.06 ± 0.01
Amrinone (x 10^{-5}M)			
1.1 ± 0.1	9.8 ± 0.8	0.25 ± 0.02	0.047 ± 0.01
2.6 ± 0.2	23.8 ± 1.5	0.22 ± 0.02	0.096 ± 0.02
6.5 ± 0.8	51.1 ± 1.8	0.21 ± 0.02	0.082 ± 0.02
26.2 ± 1.9	74.0 ± 1.7	0.21 ± 0.04	0.081 ± 0.02
70.1 ± 13.9	97.2 ± 0.9	0.23 ± 0.02	0.084 ± 0.01
IBMX (x 10^{-7}M)			
9.0 ± 1.9	7.6 ± 0.6	0.22 ± 0.01	0.045 ± 0.01
35.8 ± 6.1	26.5 ± 1.8	0.23 ± 0.01	0.08 ± 0.02
89.3 ± 32	50.6 ± 1.5	0.35 ± 0.04	0.10 ± 0.03
259 ± 55	73.8 ± 0.7	0.39 ± 0.05	0.11 ± 0.02
990 ± 177	96.4 ± 1.6	0.44 ± 0.06	0.15 ± 0.02

IBMX caused concentration-dependent increases in the levels of both cyclic nucleotides which correlated well ($r = 0.98$) with the relaxation of the vascular smooth muscle. In contrast, at concentrations that produced relaxation, amrinone increased cyclic GMP levels but not cyclic AMP levels. There exists, however, no clear correlation between the concentration of amrinone and the cyclic nucleotide levels.

The relationship between cyclic nucleotide levels and relaxation supports a mechanism of action involving the cyclic nucleotides in the vasodilator response to IBMX. In the case of amrinone, however, no such relationship is evident and its vasodilator activity may be more related to a Ca^{2+} -blocking effect (Meisheri et al. 1980).

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We would like to acknowledge the gift of amrinone from Sterling-Winthrop.

Alousi, A.A. et al (1979) Circ. Res. 45, 666.
Meisheri, K.D. et al (1980) Eur J. Pharmac. 61, 159.

ANTIHYPERTENSIVE EFFECT OF NISOLDIPINE (BAY K 5552), A NEW CALCIUM ANTAGONIST

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Vasodilators have an established place in the therapy of severe hypertension. Recently, evidence has accumulated that calcium antagonists like nifedipine are highly effective as antihypertensive drugs.

Nisoldipine (NS), a calcium antagonist with a specific action on peripheral vessels (Kazda et al., 1980), is presented here as an antihypertensive substance with a markedly enhanced efficacy, if compared with other vasodilators, namely nifedipine (NF), minoxidil (M), and hydralazine (H).

Arterial blood pressure (BP) was determined in beagle dogs of either sex (10 - 20 kg). They were supplied with an aortic catheter. Renal blood flow was reduced by 70 - 80 % by artificial renal artery stenosis to produce experimental hypertension. Hemodynamic parameters were monitored continuously in unrestrained dogs using a radio-telemetry system (Hellige, FRG). Data were processed by means of an on-line-data-acquisition system (Das 10/4; i.f.d., FRG). All drugs were administered orally.

Table 1 Antihypertensive effect of several vasodilators in unrestricted, renal hypertensive dogs (n = 4 - 5 exps. per dose)

(% decrease in mean BP)

Dose (mg/kg)	nisoldipine	nifedipine	minoxidil	hydralazine
0.0315	9.7 ± 4.1	-	-	-
0.1	13.0 ± 3.0	-	13.4 ± 4.0	-
0.315	26.5 ± 3.3	9.0 ± 2.5	36.3 ± 4.2	-
1.0	39.3 ± 6.1	14.8 ± 1.9	40.8 ± 4.5	8.7 ± 2.4
3.15	-	25.3 ± 2.9	-	12.4 ± 6.6
10.0	-	-	-	17.9 ± 4.6
ED_{20}	0.135	1.677	0.135	10
(Conf. lim.)	(0.07-0.24)	(1.08-3.52)	(0.04-0.23)	-

Pre-drug levels of mean BP were 132.8 ± 3.6 (NS), 126.4 ± 3.5 (NF), 131.3 ± 4.8 (M), and 142.9 ± 3.0 (H). NS produced a dose-dependent drop in mean BP in the dose-range examined. With respect to its ED_{20} it is about equipotent with M, approx. 10 times more active than NF, and more than 74 times more active than H (Table 1). The duration of the antihypertensive action of NS is markedly prolonged in comparison with NF. Whereas heart rate is increased for the entire period of BP depression after NF, M, and H, after NS it is increased only initially.

The results presented indicate that NS is more potent than NF and H. Reflex tachycardia after NS is less persistent than after the other vasodilators. The long-standing antihypertensive action of NS is in good agreement with its long duration of action on the isolated rabbit aortic strip (Kazda and Towart, 1982).

Kazda, S. et al (1980) Arzneim. Forsch. 30, 2144 - 2162
Kazda, S. and Towart, R. (1982) This meeting

THE DURATION OF ACTION OF CALCIUM ANTAGONISTS IN VITRO: A COMPARISON OF NIFEDIPINE AND NISOLDIPINE (BAY K 5552)

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Organic calcium antagonists are potent inhibitors of potassium-induced contractions of isolated vascular smooth muscle. This may be attributed to their blocking of potential sensitive calcium influx channels (Fleckenstein, 1977). There have, however, been few studies of their duration of action. We have used an automated system to compare the duration of action of equieffective concentrations of nifedipine and nisoldipine (BAY K 5552, see Kazda et al, 1980).

Rabbit aortic strips (Furchgott and Bhadrakom, 1953) were suspended in oxygenated Tyrode's solution. An automated control system (Towart, 1981) added KCl (final bath concentration 50mM), and after 16 minutes washed four times. This was repeated every hour and reproducible contractions could be induced for about 15 hours, after which the amplitude of contraction fell markedly.

Preincubation for 30 minutes with 2.9×10^{-7} mol/l nifedipine or with 2.6×10^{-7} nisoldipine inhibited contractions by 95% $\pm$ 2% (n=4) and 81% $\pm$ 9% (n=5) respectively. Contractions of control strips treated with the corresponding concentration of the solvent, DMSO, were not affected. Washing and incubation in drug-free solution restored the contractions of the nifedipine-treated strips with a half time of 3.5 hours. Contractions of strips tested with nisoldipine remained inhibited for 15 hours, despite washing.

Nisoldipine is similar in structure to nifedipine, but has a larger ester group in the 3 position of the dihydropyridine ring, making the molecule more lipophilic. We are investigating other such compounds to examine whether the duration of action of calcium antagonistic dihydropyridines is correlated with the lipophilicity of the molecule.

Fleckenstein, A. (1977) Ann.Rev.Pharmacol.Toxicol. 17, 149-166.

Furchgott, R.F. and Bhadrakom, S. (1953) J.Pharmacol.exp.Ther. 108, 129-143.

Kazda, S., et al. (1980) Arzneim.Forsch. 30, 2144-2162.

Towart, R. (1981) N.S.Arch.Pharmacol. 316, R37.

ANTAGONISM OF Ba^{++} AND Ca^{++} INDUCED CONTRACTIONS OF RAT ISOLATED AORTA BY DIAZOXIDE AND TOLMESOXIDE

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One method of assessing the importance of a direct interference with calcium availability by vasodilators in vascular muscle is to determine their effect on calcium (Ca^{++}) induced contractions of potassium depolarized preparations. One limitation of this technique is that Ca^{++} concentrations above 20 mM invariably cause relaxation, presumably due to membrane stabilization (Kreye et al, 1975). Wohl et al (1967) used barium (Ba^{++}) as a "convenient and potent anolog of calcium" and demonstrated a competitive antagonism of contraction of rat aorta with diazoxide. On the other hand Rhodes & Sutter (1971) using Ca^{++} on the rabbit anterior mesenteric vein demonstrated a non-competitive antagonism by diazoxide.

We have compared the effects of Diazoxide and Tolmesoxide as antagonists of contractions of rat aorta induced by Ba^{++} or Ca^{++}. Helical strips of rat descending thoracic aortae were suspended under 1 g tension in Krebs bicarbonate, gassed with 95% O_2 in CO_2 and maintained at 37°C. In those experiments where Ca^{++} was the agonist, Ca^{++} -free Krebs to which had been added 0.025 mM EGTA and 40 mM KCl was used as the bathing solution.

Following 2 hr equilibration, cumulative dose/response curves were obtained to the agonists; Ca^{++} in the dose range 0.1 to 12.8 mM and Ba^{++} in the dose range 0.02 to 15 mM. Both diazoxide (10 µM to 0.5 mM) and Tolmesoxide (10 µM to 1.0 mM) caused a parallel shift of the log dose response curve for Ba^{++} with around 20% reduction of the maximum responses seen only with the highest concentrations of antagonist used. The PA_2 for Diazoxide was 5.34 ± 0.07 (mean ± s.e.mean, n=12) and that for Tolmesoxide was 5.10 ± 0.14 (n=13). Using Ca^{++} as the agonist, both diazoxide and Tolmesoxide caused some parallel displacement of the curves with the lowest concentrations used (10 µM and 100 µM respectively). As the concentration of the antagonists was increased (up to a maximum of 1.0 mM and 10 mM respectively) the slope of the curves and the maximum obtainable contractions were reduced. The PA_h (negative log of the molar concentration of antagonist which reduces the maximum to one half; Schild 1947) for Diazoxide was 4.00 ± 0.12 (n=6) and that for Tolmesoxide was 3.05 ± 0.10 (n=6).

These results clearly indicate that a demonstration of competitive blockade of Ba^{++} contractions on rat aorta does not necessarily infer that a competitive relationship exists for Ca^{++}. Furthermore, comparative potencies may differ between agonists. Using Ba^{++} as the agonist, diazoxide and tolmesoxide appear approximately equipotent. Using Ca^{++} as the agonist, tolmesoxide was approximately ten times less potent than diazoxide.

Kreye, V.A.W. et al (1975) Naunyn-Schmiedeberg's Arch. Pharmacol. 288, 381
Rhodes, H.J. & Sutter, M.C. (1971) Can. J. Physiol. Pharmacol. 49, 276
Schild, H.O. (1947) Pharmacol. Rev. 9, 242.
Wohl, A.J. et al (1967) J. Pharmac. exp. Ther. 158, 531

MODE OF INHIBITORY ACTION OF ATP ON VASCULAR SYMPATHETIC NEUROEFFECTOR TRANSMISSION

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Adenine nucleosides and nucleotides modify adrenergic neuroeffector transmission in many tissues (Burnstock, 1981) including rabbit pulmonary artery (Husted & Nedergaard, 1981). Adenosine-5'-triphosphate (ATP) and adenosine-5'-diphosphate (ADP) release prostaglandins in various tissues (Needleman et al., 1974). Prostaglandins can inhibit the release of noradrenaline evoked by sympathetic nerve stimulation (Starke, 1977). In the present work, we studied the possibility that ATP in part inhibits stimulation-evoked release of transmitter from postganglionic sympathetic nerves by releasing prostaglandins.

The rabbit isolated pulmonary artery preparation was used. The adrenergic neurones in the arteries were excited by electrical-field stimulation (150 monophasic pulses; 0.5 msec; 3 Hz; 250 mA) and the resultant isometric contractions were recorded. The methods have been described in detail (Nedergaard, 1973).

ATP (10^{-6} - 3 x 10^{-4}M), the degradation-resistant ATP-analoque, β,γ-methylene-5'-triphosphate (APPCP; 10^{-5} - 3 x 10^{-4}M), ADP (10^{-6} - 3 x 10^{-4}M) adenosine-5'-monophosphate (AMP; 10^{-6} - 3 x 10^{-4}M), adenosine (10^{-5} - 3 x 10^{-4}M) and 2-chloroadenosine (10^{-7} - 3 x 10^{-4}M) reduced the contractions evoked by field-stimulation of the pulmonary artery. This was also the case for prostaglandin E_2 (3 x 10^{-9} - 3 x 10^{-7}M) while prostaglandin $F_{2\alpha}$ (1.4 x 10^{-10} - 3 x 10^{-8}M) slightly augmented the neurogenic response.

The time course of the inhibitory effect of purinergic compounds on the stimulation-evoked contractions of pulmonary artery was studied. In the case of ATP and ADP the inhibition was biphasic: an initial marked block (1 min after ATP addition) which in the continued presence of ATP recovered partially 10 min later and then remained almost constant for another 90 min. The other purinergic agents caused a monophasic reduction, i.e. the degree of inhibition was the same 1 and 11 min after drug addition. In the presence of the prostaglandin synthetase inhibitor indomethacin (5 x 10^{-5}M), ATP and ADP also reduced the neurogenic contractions in a monophasic manner. Indomethacin (5 x 10^{-5}M) did not alter the APPCP-induced inhibition. Dilazep (3 x 10^{-6}M) plus deoxycoformycin (3.6 x 10^{-6}M), inhibitors of adenosine uptake and deamination, respectively, augmented the inhibitory effect of ATP (10^{-7} - 5 x 10^{-5}M). In contrast, theophylline (5 x 10^{-5}M) did not alter the effect of ATP. The inhibitory effect of ATP (10^{-4}M) on stimulation-evoked contractions was inversely proportional to the extracellular Ca^{2+} concentration (0.3 - 5.2 mM) and to frequency of field-stimulation (3-15 Hz).

These results suggest that ATP initially causes a presynaptic inhibition of noradrenaline release evoked by field-stimulation. The phase I block is in part due to an ADP-mediated short-lasting release of prostaglandins of the E type. The continuous inhibition (phase II) is due to ATP and its metabolites, mainly adenosine. The phase II inhibition may possibly involve a decreased entry of Ca^{2+} into adrenergic nerve terminals during depolarization.

Burnstock, G. (1981) J. Physiol. 313, 1-35.
Husted, S. & Nedergaard, O.A. (1981) Acta pharmacol. toxicol. 49, 334-353.
Nedergaard, O.A. (1973) Eur. J. Pharmacol. 23, 153-161.
Needleman, P. et al (1974) J. Pharmacol. exp. Ther. 188, 453-460.

HUMAN UMBILICAL ARTERY IS CONTRACTED BY OXYGEN, KCl OR 5-HT BUT NOT BY α-ADRENOCEPTOR AGONISTS

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Alpha-adrenoceptors which mediate contraction of vascular smooth muscle, in vivo, comprise at least two groups (McGrath, 1982). Alpha-1 is predominant in responses to sympathetic nerve stimulation while alpha-2 and alpha-1 act together in the response to circulating catecholamines. Since respiratory acidosis favours a switch from alpha-1 to alpha-2 activation (Flavahan & McGrath, 1981) it seemed of interest whether alpha-2-adrenoceptors might be found in human umbilical artery, which is constantly exposed to acidic, de-oxygenated blood and, being non-innervated, might lack alpha-1 adrenoceptors. Furthermore, at birth, the blood in human umbilical artery has a higher than normal concentration of noradrenaline (NA). Thus a further possibility arises: that alpha-agonism might contribute to the post-natal constriction of the vessel and, when breathing starts, this might be modulated by the increase in pO2 or pH.

Umbilical arteries were dissected immediately following delivery and carried to the laboratory in cold Krebs' bicarbonate solution. Isometric tension was recorded, within 30 min, in vitro at 37°C. The Krebs' solution was bubbled with: O_2, 3.5-4.8%; CO_2, 6-7%; balance N_2. This mimics the gas tensions measured in the human umbilical artery. Viability was established as contraction to KCl, 20 mM.

5-hydroxytryptamine (5-HT) produced contraction with a threshold at 1-10 nM. Contraction to 5-HT, $\geq$ 1 μM, was larger than to any other stimulus tested. Rauwolscine (1-10 μM) antagonised the response to 5HT.

The effect of high O_2 tension was assessed by changing the gas to: O_2, 95% CO_2, 5% this produced a sub-maximal contraction but, despite the elevated baseline, did not affect responsiveness to 5HT or to KCl (10-40 mM). The contraction to O_2 was abolished by indomethacin (10 μM, 40 min).

NA was tested at physiological levels (10-300 nM) but the threshold for contraction was $\geq$ 10 μM and the contraction to 100 μM was less than that to 5-HT (100 nM) or KCl (20 mM). The synthetic, alpha-adrenoceptor agonists, amidephrine (10 μM) (alpha-1) or xylazine (100 μM) (alpha-2) produced no contraction.

These results indicate no significant population of alpha-adrenoceptors (alpha-1 or alpha-2) in the human umbilical artery. A 5-HT receptor is, however, present and it may be through this that NA exerts its small effect at high concentrations. Previous hypotheses concerning alpha-adrenoceptors in this tissue may be incorrect. For example, cocaine-induced supersensitivity (Reiffenstein & Triggle, 1974) may not involve alpha-adrenoceptors but rather 5HT receptors at which cocaine can act (Fozard et al, 1977). At concentrations found in the fetal circulation, however, we find no evidence that NA could constrict the human umbilical artery in the presence of low or high O_2 tension. Oxygen itself can however constrict the vessel, apparently through prostaglandin formation and this may be of physiological importance. Our original postulate, that alpha-2-adrenoceptors might find the umbilical artery a congenial environment, receives no encouragement.

Flavahan, N.A. & McGrath, J.C. (1981) Br. J. Pharmac. 74, 804P.
Fozard, J.R. et al (1977) Br. J. Pharmac. 61, 130P-131P.
McGrath, J.C. (1982) Biochem. Pharmacol. in the press.
Reiffenstein, R.J. & Triggle, C.R. (1974) Can. J. Physiol. Pharmacol. 52, 687-698.

THE EFFECTS OF ICI 118551 AT β_1 - AND β_2 -ADRENOCEPTORS OF GUINEA-PIG ISOLATED TISSUES

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Recent evidence indicates that ICI 118551 is a potent antagonist at β_2-adrenoceptors (Bilski et al, 1980; O'Donnell & Wanstall, 1980). This conclusion has been arrived at largely through use of selective agonists in isolated tissues that possess a mixed population of β-adrenoceptors and/or an identifiable adrenergic innervation. We, therefore, decided to compare the effects of ICI 118551 on guinea pig tissues containing a homogeneous population of β-adrenoceptors (soleus muscle, β_2; right atrial and papillary muscles β_1) with those in airway smooth muscle (β_1 and β_2).

Isolated soleus muscles were removed from pentobarbitone-anaesthetised guinea pigs and set up as described by Waldeck (1976). The soleus muscles, right ventricular papillary muscles (electrically driven at 0.4 Hz), spontaneously beating atria and spirally-cut tracheal preparations were all suspended at 32°C or 35°C in Krebs-Henseleit solution bubbled with 5% CO_2 in O_2 and their tension responses measured by conventional means. Hypertonic KCl solution (10 - 20 mM) was used in the tracheal preparations to induce a background tone against which β-adrenoceptor-mediated relaxation could be measured. In all tissues phenoxybenzamine (10μM) was used to inhibit both neuronal and extraneuronal uptake. The β-adrenoceptor agonists used in the study were isoprenaline (non-selective) and terbutaline (β_2-selective). Cumulative concentration-effect curves for each agonist were constructed, first in the absence and then in the presence of different concentrations of ICI 118551 (after allowing the necessary 60 min equilibration period to elapse). The results are summarised in Table 1.

Table 1 Mean pA_2 values ($\pm$ s.e. mean; n values in parenthesis) for ICI 118551

Tissue	pA_2 values of ICI 118551	
	Isoprenaline as agonist	Terbutaline as agonist
Trachea (β_1 & β_2)	*7.5 $\pm$ 0.04 (28)	8.5 $\pm$ 0.09 (11)
Atria (β_1)	6.90 $\pm$ 0.04 (14)	not done
PAP (β_1)	6.87 $\pm$ 0.03 (11)	not done
Soleus (β_2)	8.39 $\pm$ 0.08 (11)	8.36 $\pm$ 0.04 (6)

* Slope of the Arunlakshana and Schild plot significantly different from unity ($P < 0.05$).

In no preparation was a reduction in the maximum response to either agonist observed in the presence of ICI 118551. The antagonist was also devoid of either intrinsic sympathomimetic activity or membrane stabilising activity over the concentration range tested (1×10^{-9}M to 1×10^{-6}M).

These data are in broad agreement with previously published work (see references cited) and support the finding that ICI 118551 is a potent, selective antagonist at β_2-adrenoceptors. Taking the ratio pA_2 (soleus)/pA_2(papillary) the selectivity factor is approximately 50-fold. The results also highlight the fact that the soleus muscle contains a homogeneous β_2-adrenoceptor population whereas the trachea does not.

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Bilski, A. (1980) Br. J. Pharmac. 69, 292P
O'Donnell, S.R. & Wanstall, J.C. (1980) Life Sci. 27, 671
Waldeck, B. (1976) J. Pharm. Pharmac. 28, 434

ROLE OF β_1-ADRENOCEPTORS IN THE INHIBITORY ACTION OF NORADRENALINE ON COAXIALLY STIMULATED GUINEA-PIG ILEUM

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Sympathomimetic agents suppress transmitter output from electrically stimulated guinea-pig ileum via activation of neuronal α_2-adrenoceptors (Paton and Vizi, 1969; Drew, 1977). Although the reduction in contractile response to electrical stimulation provides a convenient measure of this effect, the smooth muscle relaxant properties of sympathomimetics may also contribute to the suppression of the electrically-induced contraction. This study attempts to assess the relative contributions of the neuromodulator and relaxant properties of noradrenaline by examining the effects of noradrenaline on both electrically-induced and acetylcholine-induced contractions of guinea-pig ileum in the presence of specific adrenoceptor antagonists.

Coaxially stimulated guinea-pig ileum preparations (0.1Hz, 0.3ms, maximal voltage) were suspended in Krebs' solution at 37°C and gassed with 95% O_2, 5% CO_2. The tension in the muscle was monitored isometrically. Single doses of sympathomimetic agents were added to the bath during stimulation. When the effect of a particular sympathomimetic on twitch height was constant, stimulation was discontinued and acetylcholine (Ach) was added in a concentration (20-30nM) previously selected to produce a contraction matching the initial twitch height.

Noradrenaline (NA, 0.3nM - 30μM) produced a concentration-dependent reduction in twitch height (maximum reduction 90-95% at 6μM NA, n=20) and in the contraction produced by exogenous Ach (maximum reduction 50% at 0.1μM NA, n=20). Isoprenaline (Iso, 0.25nM - 0.25mM) also inhibited contractions of the ileum to Ach (n=4) and the inhibitory effects of both NA and Iso on Ach-induced contractions were antagonised by propranolol (0.4 - 4μM, n=12, $p<0.05$). Propranolol (1.2μM) also antagonised the inhibition of twitch height produced by the lower concentrations of NA (up to 0.3μM, n=4, $p<0.05$) and Iso (up to 2.5μM, n=4, $p<0.05$). Similarly, atenolol (0.1 - 1μM) reduced the inhibitory effects of low concentrations of NA on both the twitch response and the response to exogenous Ach (n=8, $p<0.05$).

Prazosin (10 - 100nM) had no significant effect on either the inhibition of the twitch response or the suppression of Ach contractions by NA (n=8) but antagonised a contraction evoked by phenylephrine (3μM - 3mM, n=4). Yohimbine (50-100nM) antagonised the inhibition of twitch response produced by higher concentrations of NA (30nM - 10μM, n=8, $p<0.05$) and, at 100nM, reduced the inhibitory effect of NA on Ach-induced contractions (n=4, $p<0.05$).

It is concluded that the reduction in twitch response produced by low concentrations of NA is mediated predominantly by β_1-adrenoceptor-induced relaxation of longitudinal smooth muscle, presumably resulting in a physiological antagonism of endogenously released Ach. The remainder of the inhibitory effect of NA on the twitch response appears to be mediated by α_2-adrenoceptors, activation of which decreases Ach release from the nerve endings (Paton & Vizi, 1969) and also results in direct relaxation of ileal muscle.

Drew, G.M. (1977) Br. J. Pharmac. 59, 513P
Paton, W.D.M. and Vizi, E.S. (1969) Br. J. Pharmac. 35, 10-28

A STUDY WITH 'SELECTIVE' α_2-ADRENOCEPTOR ANTAGONISTS IN THE RABBIT VASCULATURE

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We have recently reported experiments which sought to elucidate the role of post-junctional α_1- and α_2-adrenoceptors in the vasculature of pithed rabbits (McGrath & McKean, 1981). These experiments outlined the effect of circulating catecholamines and sympathetic nerve stimulation, and the effect upon these of the 'selective' α_1-adrenoceptor antagonist, prazosin and the 'selective' α_2-adrenoceptor antagonist, rauwolscine.

It was of particular interest that rauwolscine produced a greater inhibition than did prazosin at low doses of noradrenaline (<1µg/kg) whilst the reverse was true at higher doses of the agonist (>1µg/kg). Rauwolscine was able to reduce responses to low frequency nerve stimulation (≤0.5Hz) but its effect at higher frequencies (≥1Hz) was variable. Prazosin produced a further blockade of the response at all frequencies when given after rauwolscine. These results suggested that both α_1- and α_2-adrenoceptors are involved in the response to circulating noradrenaline and in the response to sympathetic nerve stimulation.

It has been shown in vitro (Weitzell et al, 1979; McGrath, 1981) that rauwolscine possesses α_1-adrenoceptor antagonist properties. In order to verify our previous conclusion, we have used, under the same conditions, the α_2-adrenoceptor antagonist RS21361, which is more "selective" for α_2-adrenoceptors than is rauwolscine (Michel & Whiting, 1981). This compound is a benzodioxan derivative whereas rauwolscine is a yohimbine stereoisomer.

Male New Zealand white rabbits were decerebrated and pithed (McGrath & MacKenzie, 1977), carotid arterial pressure was monitored and drugs injected via a jugular vein. The diastolic pressor responses to noradrenaline and to sympathetic nerve stimulation at T8 (20 pulses, 5 msec, supramaximal voltage and 0.1-10Hz) were measured.

RS21361 (up to 10 mg/kg) was less effective against noradrenaline than was rauwolscine (1mg/kg). However, like rauwolscine, RS21361 was more effective against low doses of noradrenaline (≤1µg/kg) than against higher doses (>1µg/kg). Prazosin produced a further blockade when administered after RS21361.

Against low frequencies of nerve stimulation (≤0.5Hz) RS21361 (10mg/kg) was as effective an antagonist as was rauwolscine (1mg/kg). A combination of RS21361 and rauwolscine produced no further blockade of the responses. At higher frequencies (≥1Hz) the effect of RS21361 on the responses was variable, as was found with rauwolscine, possibly due to interference with an α_2-mediated feedback control mechanism. The effect of prazosin after RS21361 was similar to its effect after rauwolscine.

The use of a compound from a second group of 'selective' α_2-adrenoceptor antagonists, chemically distinct from the yohimbine group and the observation of the similar actions of the two groups, confirms our previous suggestion that post-junctional α_2-adrenoceptors are involved in the responses to sympathetic nerve stimulation as well as to circulating noradrenaline in this preparation.

McGrath, J.C. (1981) Br.J.Pharmac. 72, 527P-528P.
McGrath, J.C. & McKean, C.E. (1981) Br.J.Pharmac. 74, 846P-847P.
McGrath, J.C. & MacKenzie, J.E. (1977) Br.J.Pharmac. 61, 199-212.
Michel, A.D. & Whiting, R.L. (1981) Br.J.Pharmac. 74, 255P-256P.
Weitzell, R. et al (1979) Naunyn-Schmiedeberg's Arch.Pharmac. 308, 127-136.

THE INFLUENCE OF AGENTS ACTING SELECTIVELY AT α_2-ADRENOCEPTORS ON RESPIRATION IN THE RAT

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A report by Bolme et al (1977) demonstrated that the α_2-adrenoceptor agonist clonidine altered the respiratory pattern in the anaesthetized rat. The present study develops the investigation of α_2-adrenoceptor mediated effects on respiration in anaesthetized and conscious rats using the more selective α_2-adrenoceptor agonist guanabenz (Doxey et al 1981) and the α_2-adrenoceptor antagonists yohimbine and the highly selective RX 781094 (Chapleo et al. 1981).

Male Sprague-Dawley rats (200-250g) were anaesthetized with pentobarbitone (60 mg/kg, i.p.), the trachea cannulated and Fleisch pneumotachograph attached allowing respiratory rate and minute volume to be monitored using a Hewlett-Packard respiratory analyser. Drugs were administered and blood samples withdrawn for blood gas analysis (IL 613) via a carotid artery cannula. Conscious rat studies utilised rats with previously implanted aortic cannulae (Weeks and Jones, 1960).

Table I. The effects of α_2-adrenoceptor agonists and antagonists on the respiration of the conscious and anaesthetized rat (n=5).

Drug (mg/kg,i.a.) (as salt)	Anaesthetized $\Delta\%PaCO_2$	Resp.Rate	Min.Vol.	Conscious $\Delta\%PaCO_2$
Clonidine (0.1)	20±3	-34±2	-23±3	13±5
Guanabenz (0.3)	20±5	-35±2	-25±3	11±5
Yohimbine (2)	- 3±4	2±3	5±3	-10±2
RX 781094 (1)	4±4	- 7±1	0±7	- 7±7
Yohimbine (2)+ clonidine(0.1)	12±1	20±2	16±2	- 4±3
RX 781094 (1) + clonidine(0.1)	- 9±5	3±4	16±3	5±6

Absolute control values: 49±2 mmHg ($PaCO_2$ anaesthetized rats),93±9 breaths/(Resp.Rate), 300±20mL (min.vol.) and 33±0 mmHg ($PaCO_2$ conscious rats).

The results summarised in Table I show that both clonidine and guanabenz depress respiratory rate and minute volume and increase the $PaCO_2$. Whilst yohimbine and RX 781094 themselves had little effect on respiration they at least partly (yohimbine) or completely (RX 781094) antagonised the effects of clonidine.

In conclusion, the data presented indicates that α_2-adrenoceptor activation produces a depression of the mechanics of respiration leading to a significant reduction of gaseous exchange. α_2-adrenoceptor antagonists themselves were without much effect indicating a low level of α_2-adrenoceptor influence on basal maintenance of gaseous exchange.

Bolme P. et a. (1977) Eur.J.Pharmac. 28, 89-94
Chapleo C.B. et al (1981) Br.J.Pharmac. 74, 842P
Doxey J.C. et al. (1981) J.Auton.Pharmac. 1, 157-169
Weeks, J.R. and Jones,J.A.(1960) Proc.Soc.Exper.Biol. and Med.104, 646-648

SELECTIVITY OF SUBSTITUTED BENZOQUINOLIZINES FOR α_2-ADRENOCEPTORS IN PITHED RATS

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The selectivity of a series of novel substituted benzoquinolizines as antagonists at alpha$_2$ adrenoceptors has previously been evaluated in rat isolated tissues (Lattimer et al, 1981). The purpose of the present study was to evaluate the antagonist activity of these compounds at alpha$_2$ and alpha$_1$ adrenoceptor sites in the intact animal. Their activities have been compared with that of the selective alpha$_2$ adrenoceptor antagonist yohimbine and with indoramin, a drug showing selectivity for alpha$_1$ adrenoceptors (Algate and Waterfall, 1978).

Rats were anaesthetised (5% halothane in O_2) pithed and artificially respired (60 strokes /min; 1ml/100g). Blood pressure was recorded from the left femoral artery and drugs were administered via a cannula in the left femoral vein. All animals received tubocurarine (1mg/kg i.v.).

For presynaptic measurements the pithing rod was used to stimulate the spinal outflow (Armstrong and Boura, 1973) and constant responses to stimulation (0.5ms, 25V; 1Hz for 1 min) were obtained after which clonidine (0.25-512µg/kg) was administered cumulatively at intervals of 5 min. The stimulation was repeated 0.5 min after each dose. Experiments were made in the presence of yohimbine, Wy 24965, Wy 25309, Wy 26392, Wy 26703, indoramin or vehicle, administered 3 min before the first dose of clonidine.

In separate experiments the alpha$_1$ antagonist actions were determined against phenylephrine-induced vasopressor responses. Response curves to phenylephrine were made before and 3 min after adminstration of the same doses of compounds.

Shifts to the right of the control dose response curves for clonidine induced inhibition of tachycardia and phenylephrine induced pressor responses were obtained for each antagonist. ED_{50} dose ratios and a selectivity index, derived by subtracting 1 from each ED_{50} dose ratio and dividing, are shown in Table 1.

Table 1 ED_{50} dose ratios at alpha$_2$ and alpha$_1$ adrenoceptor sites

Compound	Dose	ED_{50} dose ratio ± S.E.M. Alpha$_2$	Alpha$_1$	Selectivity index
Indoramin	3.2	1.9 ± 0.7	8.6 ± 2.3	0.1
Yohimbine	1	20.9 ± 2.3	4.0 ± 0.9	6.6
Wy 24965	1	6.4 ± 0.4	3.4 ± 1.0	2.3
Wy 25309	1	15.7 ± 2.3	2.2 ± 0.2	12.3
Wy 26392	0.3	13.4 ± 2.4	1.6 ± 0.2	21.0
Wy 26703	0.3&1.0*	13.6 ± 4.5	1.39± 0.13	≈100.0

* Doses are for Alpha$_2$ and Alpha$_1$ sites respectively.

Indoramin showed selectivity for the alpha$_1$ adrenoceptor site in keeping with previous studies made in pithed rats (Algate and Waterfall, 1978). Wy 24965, Wy 25309, Wy 26392, Wy 26703 and yohimbine were selective for the presynaptic (alpha$_2$) site. Wy 25309, Wy 26703 and Wy 26392 were more selective than yohimbine. Compounds of this type may be useful in the further elucidation of the physiological role of alpha2 adrenoceptors.

Algate, D.R. & Waterfall, J.F. (1978) J. Pharm. Pharmac. 30, 651-652.
Armstrong, J.M. & Boura, A.L.A. (1973) Br. J. Pharmac. 47, 850-852.
Lattimer, N. et al (1981) Br. Pharmac. Soc. Meeting, London.

CONTRACTIONS OF ANTRAL CIRCULAR SMOOTH MUSCLE ARE MEDIATED VIA ATROPINE AND YOHIMBINE SENSITIVE MECHANISMS

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Exogenous catecholamine treatment can cause contraction and relaxation of circular smooth muscle strips taken from the antral region of guinea pig stomach (Sahyoun et al, 1981). In the present experiments we attempt to determine whether the effects induced by exogenous catecholamine are reflective of neurotransmitter release by analysis of the effects of field stimulation of antral circular smooth muscle.

Preliminary experiments indicated that a change in muscle tension to field stimulation could be secured by employing pulse durations in excess of 0.1 ms, 20V across the tissue and frequencies of stimulation in excess of 0.125 Hz. Field stimulation at these parameters elicited contraction, the spontaneous contractions being incorporated into the electrically induced response. Even at 20 Hz, relaxations were never recorded, although at 10 Hz and greater field stimulation reduced spontaneous activity. Tetrodotoxin (10^{-7}M) abolished all components of the electrically induced changes in muscle tension. Atropine in nM concentrations abolished the contractile effects over the entire frequency range. After the prevention of contraction there was no evidence of frequency-related relaxation; a very small relaxation was apparent above 1 Hz (less than 10% of the size of maximum contraction), but the size of this did not increase up to 20 Hz. Prazosin (10^{-8}-10^{-6}M) and propranolol (10^{-8}-10^{-6}M) failed to modify the contractions. In contrast, yohimbine caused an approximate 50% inhibition at 10^{-8}M with further reductions apparent at 10^{-7}M and 10^{-6}M, although the contractions were never abolished. The contractions were not modified by haloperidol (10^{-8}-10^{-6}M) but were enhanced by metoclopramide (10^{-8}-10^{-6}M). The metoclopramide (10^{-7}M)-enhanced contractions were not antagonised by yohimbine (10^{-6}M) but were abolished by atropine (10^{-8}M).

The ability of atropine to abolish the contractile response to field stimulation of antral circular smooth muscle suggests a major cholinergic involvement, and complicates the intention to determine the effects of sympathetic nerve stimulation. However, the marked reduction in contraction also afforded by yohimbine is suggestive of an additional sympathetic involvement at α_2-adrenoceptors. Perhaps significantly, the response most readily elicited from exogenous catecholamine treatment is one of contraction, albeit succeeded by relaxation. The nature of the cholinergic-sympathetic interaction to facilitate contraction is not clear, but the enhanced responses to metoclopramide, resistant to yohimbine, indicates that the cholinergic system operates subsequent to the sympathetic mechanism.

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Sayhoun, H.A. et al (1981). J. Pharm. Pharmac. in press.

AN INVESTIGATION INTO CROSS TOLERANCE BETWEEN KETAMINE AND MORPHINE

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It has been shown recently that the injectable anaesthetic agent, ketamine, has some opiate agonist activity (Lawrence & Livingston, 1981). This investigation has examined this activity further by testing the cross tolerance between ketamine and morphine in the rat. Tolerance to morphine was induced in groups of 6 adult male Wistar rats (300 g body weight) by twice daily (9.00 and 17.00 h) injections of morphine hydrochloride (10 mg/kg subcutaneously) for a period of nine days. Similarly, ketamine tolerance was induced by daily (9.00 h) injections of ketamine hydrochloride (50 mg/kg intraperitoneally) for nine days. Control animals in each case received similar daily injections of physiological saline.

Animals were assessed for tolerance to morphine by measuring the analgesic response to the tail flick test in water at 55^{o}C, and to ketamine by measuring the anaesthetic sleeping time using the righting reflex and also the tail flick test for analgesia.

The animals receiving repeated morphine injections were monitored daily for the analgesic response and by the ninth day there was a 50% reduction in response time showing the development of tolerance. On the tenth day these rats were injected with ketamine (100 mg/kg i.p.) and the effects on sleeping time and analgesic response compared to the saline injected controls. There was no significant difference between the two groups in either test.

The animals which had received repeated injections of ketamine were injected with morphine hydrochloride (10 mg/kg s.c.) or ketamine (100 mg/kg i.p.) on the tenth day and the anaesthetic and analgesic responses compared to the saline injected controls. The saline treated animals were more sensitive to the analgesic effects of ketamine than the ketamine treated group and showed a significantly longer sleeping time indicating that ketamine tolerance had developed. In the ketamine tolerant animals injected with morphine on day ten the maximum analgesic response was significantly reduced ($p < 0.05$) from a mean of 9.4 ± 0.8 sec in the control animals to 5.9 ± 0.9 sec in the ketamine tolerant animals.

These results suggest a one directional tolerance between ketamine and morphine which may either be associated with receptor effects, or may reflect alterations in morphine metabolism in ketamine tolerant rats.

Lawrence, D. & Livingston A. (1981) Br. J. Pharmac. 73, 435-442.

DOES REPEATED EXPOSURE TO THE NARCOTIC ANTAGONIST NALOXONE AFFECT SENSITIVITY TO THE DRUG'S EFFECT?

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The use of naloxone as a narcotic antagonist is well documented and it has been shown that this compound can strongly influence food and water intake in experimental animals (Sanger, 1981). There is only limited information on the development of tolerance to narcotic antagonists, a situation which prompted the present investitation.

Rats received two subcutaneous injections (day 1 and day 7) of a slow release vehicle containing either naloxone (100 mg/kg) or saline. On day 14 the animals were divided into separate groups. Assessment was made of the ability of acutely administered naloxone either to antagonise the analgesic effect of morphine (10 mg/kg s.c.) using a hot water nociceptive stimulus or to reduce food and water intake (McCarthy et al, 1981).

Further studies were conducted using rabbits adapted to a two hour feeding schedule. Naloxone was injected at 3mg/kg s.c. twice a week for 10 weeks and food and water intakes measured. A second group of rabbits with guide cannulae implanted into the left lateral ventricle, were injected once a week with saline followed next day with naloxone (0.1 - 30 μg) in a 50 μl dose volume. Fifteen minutes after injection food and water intakes were recorded over the 2 hr scheduled period.

The antagonist efficacy of naloxone (0.01 - 0.3 mg/kg s.c.) in the tail flick test for morphine (10 mg/kg s.c.) analgesia in rats was impaired to a significant degree by prior sustained exposure to naloxone, whilst no statistically significant effect was observed to the acute effects of naloxone on food and water intake in the same species.

Rabbits exhibited an enhanced responsiveness to the effects of repeated subcutaneous injections of naloxone on food and water intake whilst after repeated central administration, in otherwise identical conditions, the efficacy of naloxone was severely attenuated.

The decreased sensitivity to naloxone observed in the naloxone pretreated rats on antinociceptive testing may be attributable to an increase in the sensitivity to morphine although this was not apparent from the tail flick latencies of the vehicle and naloxone pretreated groups after morphine administration. The lack of effect of the pretreatment on the sensitivity of food and water intake to acute naloxone may reflect distinctions in the accessibility of the receptors or possibly in the nature of the opiate receptors involved in the two tests.

The effects after repeated systemic or central injections in rabbits may indicate a physiological or pharmacological distinction between central and peripheral opiate receptors with respect to food and water intake.

McCarthy, P.S. et al, (1981). Neuropharmacology, in press.
Sanger, D.J. (1981), Appetite, 2, 193-208.

EFFECTS OF SKF 10,047 COMPARED WITH OTHER OPIATES IN VARIOUS NOCICEPTIVE TESTS

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Martin et al. (1976) who characterized the benzomorphan analogue SKF 10,047 (N-allylnormetazocine) as a putative σ opiate agonist reported it to weakly depress the flexor reflex in the dog. This was not enforced by the observation that SKF 10,047 was devoid of agonist activity in phenylquinone writhing (Pearl and Harris 1966) and hot plate (Ward et al. 1978) tests using mice. In the present study we have evaluated and compared the activity of subcutaneous injections of SKF 10,047 with morphine (putative μ), ethylketocyclazocine (putative κ) and pentazocine in rodents utilizing a number of nociceptive methods. For this purpose the tail immersion (55°C) and tail pressure tests in rats, plus the tail immersion (48°C), hot plate (55°C) and acetic acid (3%)-induced abdominal writhing tests in mice were employed.

Morphine and ethylketocyclazocine produced dose related increases in response latencies in all thermal and mechanical tests in both species with steep dose response lines. In contrast, pentazocine was similarly active but exhibited a ceiling effect at doses of 20mg kg^{-1} and higher with much shallower dose response curves. However, in the acid-induced abdominal writhing test dose-related effects were observed, 100% protection being achieved at the higher doses of all three agents. SKF 10,047 at doses ranging from 0.5 to 20mg kg^{-1} in the writhing test and 2.5 to 40mg kg^{-1} in mouse hot plate, and rat tail immersion tests failed to produce any significant change in either the degree of writhing or response latencies. However in the mouse tail immersion test SKF 10,047 produced significant elevation of response latencies, the dose response line being shallow. Maximal activity was observed at 10mg kg^{-1} above which ceiling effects were noted (similar to pentazocine). However its activity displayed a comparatively slow onset and relatively long duration of action, the peak effect occurring at 60 min post injection. This antinociceptive activity was stereospecifically blocked by the opiate antagonists Mr-1452 (2.0mg kg^{-1} i.p.) and Mr-2266 (2.0mg kg^{-1} i.p.). This observation accords with the concept that SKF 10,047 interacts with opiate receptors and the similarity of its action profile to pentazocine in the tail immersion test, which is sensitive to opiate partial agonists (Sewell and Spencer, 1976) suggests that SKF 10,047 possesses some partial agonist properties itself. Consequently, this may explain why SKF 10,047 is virtually devoid of activity in most other nociceptive tests. However, our data does not allow speculations to be drawn as to the type of receptor(s) involved since SKF 10,047 has been shown to possess high affinity for both putative μ and σ receptors (Martin et al., 1976).

Martin et al. (1976) J. Pharmacol. Exp. Ther. 197 517-532.
Pearl, J. and Harris, L.S. (1966) J.Pharmacol. Exp. Ther. 154 319-323.
Sewell, R.D.E. and Spencer, P.S.J. (1976) Neuropharmacology 15 319-323
Ward, S.J., Metcalf, G. and Rees, J.M.H. (1978) in: Characteristics and functions of opioids. Van Ree, J.M. and Terenius, L. (eds) pp. 497-498, Elsevier/North Holland.

INTERACTIONS OF OPIATE κ-RECEPTOR AGONISTS WITH OPIATE ANTAGONISTS ON THE RABBIT ISOLATED VAS DEFERENS

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Previous data (Oka et al. 1981; Römer et al. 1981), indicate that the electrically-stimulated isolated rabbit vas deferens (RVD) may represent a specific assay preparation for ethylketazocine (EKZ)-like compounds. Since such an in vitro preparation would be useful in separating the actions of different types of opiates, the interactions of 3 EKZ-like compounds with the opiate antagonists naloxone and Mr 2266 have been studied. The stereospecificity of the effect was checked using the (+) and (-)-isomers of bremazocine.

The method used was essentially the same as that described by Oka et al. (1981). The epididymal and prostatic halves of the vasa deferentia from New Zealand rabbits (ca.3 kg) were suspended with a resting tension of 200 mg in 10 ml baths containing oxygenated Krebs solution at 37°C. Longitudinal contractions were recorded isotonically (Harvard 368A transducer). 4-6 tissues were used for each concentration of antagonist.

All 3 agonists caused a conc.-dependent inhibition of the RVD. The (-)-isomer of bremazocine (IC50 = 1.6 nM) was 4 times more potent than the racemate, whereas the (+)-isomer was inactive (IC50 ›› 2.5 μM). Both antagonists produced parallel displacements of the conc. response curves of the agonists. Comparison of the mean IC50's of the agonists in the presence of the antagonists showed that Mr 2266 was 2-4 times more potent an inhibitor than naloxone (Table 1).

The results show that the RVD exhibits stereospecific selectivity to EKZ-like opiates and indicate that their effects are more sensitive to inhibition by an opiate kappa-receptor antagonist than by an opiate mu-receptor antagonist.

Table 1 Mean IC50's of 3 EKZ-like compounds in the RVD in the absence and presence of opiate antagonists

Test Substance	Without Antagonist	IC50 nM (± S.E.) + Naloxone (nM) 10	20	40	+ Mr 2266 [1] (nM) 5	10	20
Ketazocine	54 (7.9)	48 (10.7)	40 (7.1)	102 (10.5)	39 (4.8)	84 (13.1)	147 (14.5)
Mr 2034 [2]	23 (2.2)	28 (2.8)	23 (3.3)	42 (1.5)	34 (4.8)	45 (4.9)	58 (4.9)
Bremazocine	6.4 (1.0)	6.4 (1.8)	8.6 (2.2)	20 (2.9)	2.0 (0.2)	10.8 (4.9)	14.5 (1.5)

1) (-)-5,9α-Diethyl-2-(3-furylmethyl)-2'-hydroxy-6,7-benzomorphan

2) (-)-(1R,5R,9R,2"R)-5,9-Dimethyl-2'-hydroxy-2-tetrahydrofurfuryl-6,7-benzomorphan (both from Boehringer, Ingelheim, GFR)

Oka, T. et al. (1981). Europ. J. Pharmac. 73, 235-236
Römer, D. et al (1981). Proceedings of the 3rd World Congress on Pain, Edinburgh 1981. Raven Press (in press)

EXCEPTIONAL OPIATE POTENCY OF 14β-ALKYL DERIVATIVES OF MORPHINONE AND CODEINONE IN VITRO AND IN VIVO

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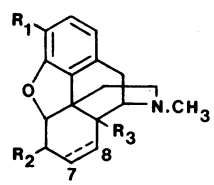
Potent antinociceptive activity has been previously described for 14β-acylated derivatives of codeinone (Buckett et al, 1964). Large cyclic or straight-chain 14β-substituents are an important key to opiate agonist potency, with the potential for development of analgesics of agonist-antagonist profile (Buckett & Bosman, 1972), although acylated compounds possess a short duration of antinociceptive action believed due to the hydrolysable 14β-function. The recent availability of 14β-alkyl morphine derivatives (Fleischhacker & Richter, 1979) allowed substantiation of the previous structure-activity relationships and investigation of duration of action.

Compounds were tested in vitro on the coaxially-stimulated guinea pig ileum as previously described (Buckett, 1979) to obtain IC50 values from five assays on tissues from at least three animals. In vivo ED50 values for antinociception were determined using groups of ten CD1 mice (20-25 g) and the tail clip method 2 min after i.v. administration.

Table 1 Opiate activity of 14β-alkyl derivatives of morphinone and codeinone

Compound					Guinea pig ileum		Mouse antinociception	
					Molar IC50 ± S.E.M.	Relative potency	ED50	Relative potency
	R_1	(7-8)	R_2	R_3	(n = 5)	(Morphine = 1.0)	mg/kg i.v.	(Morphine = 1.0)
14β-Methyldihydrocodeinone	OMe	-	=O	Me	$4.1 \pm 1.4 \times 10^{-8}$	1.8	0.02	150
14β-Ethyldihydrocodeinone	OMe	-	=O	Et	$1.3 \pm 0.1 \times 10^{-8}$	5.8	0.005	600
14β-Ethyldihydrocodeine	OMe	-	-OH	Et	$5.2 \pm 0.6 \times 10^{-8}$	1.4	0.03	100
14β-Methylcodeinone	OMe	=	=O	Me	$6.6 \pm 0.3 \times 10^{-7}$	0.62	0.34	9
14β-Ethylcodeinone	OMe	=	=O	Et	$7.7 \pm 2.1 \times 10^{-9}$	9.7	0.01	300
14β-Ethylmorphinone	OH	=	=O	Et	$6.4 \pm 0.6 \times 10^{-10}$	117	0.0003	10000
14β-Ethyldihydromorphinone	OH	-	=O	Et	$3.0 \pm 0.6 \times 10^{-9}$	25	0.0004	7500
Codeinone	OMe	=	=O	H	$1.2 \pm 0.3 \times 10^{-6}$	0.06	-	-
Morphine	OH	=	-OH	H	$7.5 \pm 0.7 \times 10^{-8}$	1.0	3.0	1.0

Introduction of a 14β-methyl group led to potent opiate activity (Table 1). Enlarging the alkyl substitution to ethyl markedly augmented the activity, with 14β-ethylmorphinone showing exceptional potency in vivo compared with morphine. The action of the compounds was reversed by naloxone (10^{-8} M) on the ileum. A significant correlation ($r = 0.87$; $P < 0.01$) was found between results from in vitro and in vivo studies and since a correlation between potency on the ileum and clinical analgesic dose exists (Kosterlitz & Waterfield, 1975) the compounds would be expected to be highly active in man. The duration of antinociceptive action of these 14β-derivatives was shorter than that of morphine. The short duration of action of analogous 14β-acyl compounds may not therefore be due to metabolism of the ester group.

Buckett, W.R. (1979) European J. Pharmac., 57, 267
Buckett, W.R. et al (964) J. Pharm. Pharmac., 16, 174
Buckett, W.R. & Bosman, H.H. (1972) Brit. Pat., 1, 300, 419
Fleischhacker, W. & Richter, B. (1979) Chem. Ber., 112, 2539 & 3054
Kosterlitz, H.W. & Waterfield, A.A. (1975) Ann. rev. Pharmac. Toxicol., 15, 29

LACK OF EFFECT OF CALCITONIN ON THE RESPONSE OF THE RAT COLON TO LEU- AND MET- ENKEPHALIN, OR ACETYLCHOLINE, IN VITRO

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Calcitonin, administered by central injection, possesses analgesic properties (Braga et al, 1978; Bates et al, 1981a) not observed when the hormone is administered peripherally. Calcitonin-induced analgesia in the mouse shares several features in common with opiate analgesia, including antagonism by calcium ions and naloxone. However, the dose of naloxone required to antagonise calcitonin-analgesia in the mouse is 10-100 fold greater than those required to antagonise opiate analgesia (Bates et al, 1981b), which suggests that calcitonin may not interact directly at the opiate receptor.

We have studied the effect of calcitonin on the rat colon, a tissue which contains excitatory opiate receptors of the μ and δ types (Boura and Olley, 1981, Gillan and Pollock, 1980). In this tissue, opiates produce a contractile response, possibly by activation of serotoninergic neurones (Huidoboro-Toro et al, 1981).

The middle third of the colon was removed from CFY rats (♂,♀, 200-300g) and suspended from an isotonic transducer under 1g tension. The tissue was superfused with warmed (37°C), gassed (5% CO_2/95% O_2) Krebs solution (Boura et al, 1981) at a rate of 4ml min^{-1}. Agonists and antagonists were injected into the superfusion fluid. Each piece of tissue was tested with acetylcholine (10^{-8}-10^{-3}M) prior to the application of the opioid peptides leu and met- enkephalin or salmon calcitonin (4700 i.u. mg^{-1}; mw = 3600). Agonist potency was determined from the EC_{50}, and the potency of the antagonists was determined by estimation of the pA_2 value.

The preparation contracted in response to acetylcholine (EC_{50} = 5 x 10^{-7}M) and the response was antagonised by atropine (pA_2 = 7.1). Both leu and met- enkephalin elicited a contractile response (leu- enkephalin EC_{50} = 3 x 10^{-8}M; met- enkephalin EC_{50} = 6 x 10^{-8}M), with a threshold concentration of 10^{-8}M and a maximum response at 10^{-6}M. The maximum response to both peptides was only 20-50% of the maximum response to acetylcholine. The response of the rat colon to the opioid peptides was antagonised by naloxone with a pA_2 value of 7.1 for leu- enkephalin and 7.0 for met- enkephalin. These results for naloxone and the maximum enkephalin response are similar to those of Boura and Olley, 1981.

Salmon calcitonin (2.5 x 10^{-12} - 2.5 x 10^{-4}M; n = 4) did not stimulate the rat colon; nor did it affect the responses to either of the opioid peptides. Similar observations have been reported by Braga et al (1978) using the transmurally stimulated guinea-pig ileum as the opiate bioassay preparation.

We conclude that calcitonin does not interact with the μ or δ opiate receptors in this preparation. Since opiate-induced analgesia in the abdominal constriction test is mediated via the μ receptor, we also conclude that the central analgesic effect of calcitonin is not mediated by direct interaction with opiate receptors of the μ type.

The salmon calcitonin was generously donated by Armour Pharmaceuticals Corporation Eastbourne, U.K.

Bates, R.F.L. (1981a) Br.J.Pharmac. 72, 575P
Bates, R.F.L. (1981b) Br.J.Pharmac. 74, 279P
Boura, A.L. and Olley, J.E. (1981) Br.J.Pharmac. 74, 246P
Braga, P. et al (1978) Life Sciences 22, 921-978
Gillan, M.G.C. and Pollock, D. (1980) Br.J.Pharmac. 68, 381-392
Huidoboro-Toro, J.P. and Way, E.L. (1981) Br.J.Pharmac. 74, 681-694.

INHIBITION OF ABDOMINAL CONSTRICTIONS BY CALCIUM ANTAGONISTS AND THEIR INTERACTION WITH CALCITONIN AND DIVALENT CATIONS

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The analgesia induced by the opiates (Chapman and Way, 1980) and calcitonin (Bates et al, 1981) may result from changes in the distribution of calcium ions within the cells of the brain. In addition, the central injection of lanthanum ion, a calcium flux inhibitor, also results in analgesia which is reversed by naloxone. However, the possible analgesic properties of the 'slow' calcium channel antagonists have not been studied. In this investigation, the analgesic properties of the calcium antagonists nifedipine and PY 108-068 have been studied.

Groups of 10 CFLP mice (♀,♂, 30g) were given intracerebroventricular (i.c.v.) injections of nifedipine, PY 108-068, salmon calcitonin (SCT), $CaCl_2$ or $MgCl_2$, dissolved in 10µl of tris-saline pH 7.4 containing 50% dimethyl sulphoxide. 10 minutes later, mice were given an i.p. injection of acetic acid (0.3ml of 1% w/v) and the frequency of abdominal constrictions was counted between the 10-14th minute following the injection of acid. In the control mice, which received an i.c.v. injection of vehicle, the rate of abdominal constrictions was 3.02 ± 0.1 min^{-1} ($\bar{x}$ ± se). Some groups of animals did not receive acetic acid, but were used to assess locomotor activity and investigative behaviour, using standard rotating drum and hole board tests respectively.

The i.c.v. injection of 0.33, 3.3 or 6.6 µmoles kg^{-1} nifedipine caused 5 ± 5, 26 ± 4* and 50 ± 2%* ($\bar{x}$ ± se, *$p<0.005$) inhibition of the frequency of abdominal constrictions. At these doses, there was not impairment of locomotor or investigative behaviour.

The simultaneous i.c.v. injection of $CaCl_2$ (1.7 µmoles kg^{-1}), together with 3.3 µmoles nifedipine, reversed the effect of the calcium antagonist. This dose of $CaCl_2$ is inactive by itself. In contrast, the simultaneous i.c.v. injection of $MgCl_2$ (3.3 µmoles kg^{-1}) together with nifedipine (3.3 µmoles kg^{-1}) did not reverse the effect of nifedipine.

Intracerebroventricular injection of SCT (0.1 or 2 i.u. kg^{-1}) inhibited the frequency of abdominal constrictions by 22 ± 4* and 49 ± 4%* ($\bar{x}$ ± se;*$p<0.005$). The simultaneous i.c.v. injection of SCT (0.1 i.u. kg^{-1} and nifedipine (0.33 µmoles kg^{-1}) produced a summation of the effects of these agents. Similar results were obtained when SCT (2 i.u. kg^{-1}) and nifedipine (3.3 µmoles kg^{-1}) were simultaneously injected i.c.v.

The i.c.v. injection of 0.33, 3.3 or 6.6 µmoles kg^{-1} PY 108-068 caused 34 ± 3*, 43 ± 6* and 57 ± 4%* ($\bar{x}$ ± se;*$p<0.005$) inhibition of abdominal constrictions. At these doses, there was no impairment of locomotive or investigative behaviour.

In summary, nifedipine and PY 108-068 given by i.c.v. injection, significantly reduce the frequency of abdominal constrictions induced by acetic acid. The effect of nifedipine was additive with that of calcitonin and, like the effect of calcitonin, could be reversed by i.c.v. injection of calcium ion but not by i.c.v. injection of magnesium ion.

The drugs used were generously donated by the following: salmon calcitonin (Armour Pharmaceutical Corp.), nifedipine (Bayer UK Ltd.) and PY 108-068 (Sandoz Ltd.).

Bates, R.F.L. (1981) Brit. J. Pharmac. 72, 575P

Chapman, D.B. & Way, E.L. (1980) Ann. Rev. Pharmacol. Toxicol. 20, 553-579.

SELECTIVE EFFECTS OF HYDROXYMAPROTILINE ENANTIOMERS ON OPIATE ANTINOCICEPTION

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We have previously studied the effects of antidepressants, alone and in combination with analgesic agents (Gonzalez et al. 1980). These studies have been extended to (+) and (-) hydroxymaprotiline (HMP), the former enantiomer possessing amine pump inhibition activity (particularly for noradrenaline (NA), Delini-Stula, personal communication). We have examined the effects of these two enantiomers in combination with morphine and pentazocine, in order to explore the possibility that analgesic enhancement is related to amine pump inhibition.

The effects of (+) and (-)-HMP alone were evaluated on nociceptive responses in the acetic acid writhing test, hot plate test (55°C) and tail immersion test (48°C) in male albino mice. Both enantiomers protected against writhing, the (+)-form displaying significantly greater activity than the (-)-form at the higher doses, there being total protection at 10mg kg^{-1} s.c. This result reflects the non-specificity of the writhing model as an antinociceptive test. In the hot plate test neither (+)- nor (-)-HMP (s.c. and i.c.v.) significantly modified reactivity to the stimulus ($P>0.05$), except for doses of the order of 100mg kg^{-1} s.c. and 50mcg icv/animal of (+)-HMP, which then interfered with reaction latencies by inducing marked sedation. Likewise, administration (s.c. or i.c.v.) of (+)- or (-)-HMP in the tail immersion test produced no significant modification of nociceptive sensitivity, although 100mg kg^{-1} s.c. of (+)-HMP produced a mild 35% antinociceptive effect. However, pretreatment (30 min) with doses up to 10mg kg^{-1} s.c. or 10mcg icv/animal of (+)-HMP significantly potentiated the antinociceptive effects of both morphine and pentazocine ($P<0.001$), and also shifted their dose-response lines to the left. Similarly (+)-HMP significantly potentiated morphine analgesia in the hot plate, suggesting an overall increase in antinociceptive potency of these opiates when combined with (+)-HMP. In contrast however, pretreatment with (-)-HMP failed to significantly modify the antinociceptive effects of either morphine or pentazocine in the tail immersion or hot plate tests.

Other investigators have studied the effects of various antidepressants on nociceptive sensitivity, and also on opiate analgesia (Saarnivaara et al. 1974; Malseed et al. 1979; Ogren et al. 1980). Saarnivaara demonstrated the ability of antidepressants, possessing differential effects on NA and 5HT reuptake, to delay the vocalisation response to electrical stimulation of tooth pulp in rabbits. Similar studies using thermal antinociceptive tests with rodents have not been undertaken previously and we report here that HMP alone does not display antinociceptive effects in either the hot plate or tail immersion test, though the literature is unified on the effects of uptake inhibitors on opiate analgesia. A range of agents has been tested, with varying specificity on NA and 5HT reuptake using different species and testing methods, and in all cases consistent potentiation of morphine analgesia has been described. The results reported here demonstrate a stereospecific effect of HMP in enhancing opiate antinociceptive effects. This would imply that a specific mechanism may mediate these synergistic effects, probably by the promotion of central NA activity, whereas the non stereospecific pharmacological actions of HMP such as antihistaminic activity (which is common to both enantiomers) might be irrelevant. These findings would add further support to the concept of a positive modulatory role for central NA pathways in the mediation or modulation of opiate analgesic effects.

Gonzalez, J.P., Lee, R.L., Sewell, R.D.E. & Spencer, P.S.J. (1980) Roy. Soc. Med. Int. Cong. Symp. 25 59-69
Malseed, R.T. & Goldstein, F.J. (1979) Neuropharmacology 18 827-829
Ogren, S.O. & Holm, A.C. (1980) J. Neural Transmission 47 253-271
Saarnivaara, L. & Mattila, M.J. (1974) Psychopharmacology 35 221-236

STUDIES ON THE ROLE OF CALCIUM IN INDOMETHACIN INHIBITION OF HISTAMINE-INDUCED SYNOVIAL VASCULAR PERMEABILITY

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Many of the pro-inflammatory actions of histamine are inhibited by non-steroidal anti-inflammatory drugs (Famaey et al, 1975), this may be a Ca^{++}-dependent action (Northover, 1975). We have used a recently developed method permitting continuous monitoring of vascular tone and permeability in the rabbit synovium (Al-Haboubi and Zeitlin, 1980) to study the action of histamine on the synovial vasculature, and its modification by indomethacin. Synovial cavities in anaesthetised rabbits were perfused, with Krebs' solution (0.1 ml/min., 37°C) alone or containing drugs, using push-pull concentric needle cannulae. Leakage of ^{125}I-albumin into the synovial perfusate was used to assess vascular permeability. External monitoring of the content of in vivo-labelled ^{99m}Tc-erythrocytes was used to monitor synovial vascular tone. Intra-articular infusion with low concentrations of histamine ($9 \times 10^{-10} - 9 \times 10^{-9}$M) decreased vascular tone with no change in vascular permeability. Increasing concentrations above 9×10^{-9}M progressively increased vascular tone towards the basal value, accompanied by increasing leakage of ^{125}I-albumin into the synovial perfusate. Histamine ($9 \times 10^{-7} - 2.5 \times 10^{-4}$M) infused in the presence of indomethacin (7×10^{-5}M) produced negligible change in vascular tone and an increase in vascular permeability less than 50% of that from histamine alone. Intra-articular infusion with the maximally effective dose of histamine (2.5×10^{-4}M) in the presence of indomethacin (7×10^{-5}M) produced a mean increase in permeability only 37.5% of that produced by this histamine concentration alone (Table 1), while producing no significant change in vascular tone. When these concentrations of histamine and indomethacin were infused in Krebs' solution containing twice (5.0 mM) the normal concentration of Ca^{++}, the mean permeability increase rose to 69.4% of that of histamine alone, with no significant change in vascular tone ($P > 0.05$). Perfusion of the synovium with histamine (2.5×10^{-3}M) in calcium-free Krebs' solution containing the calcium-selective chelating agent, EGTA (0.1 mM), produced an increase in mean vascular permeability only 11.6% of that produced by the histamine alone, and a net increase in mean vascular tone compared to that produced by EGTA in calcium-free Krebs' alone. The effect of calcium lack on histamine-induced permeability increase was not secondary to a vaso-constrictor action of EGTA, since EGTA-perfusion in calcium-free Krebs' produced no significant change in mean vascular tone compared to perfusion with normal Krebs'. These findings support a role for calcium in both the vascular response to histamine in rabbit synovium and its inhibition by indomethacin.

Krebs'	Ca^{2+}-free Krebs'	Histamine alone	Histamine + Indomethacin	Histamine + Indomethacin in Krebs' (5 mM Ca^{2+})	Histamine in Ca^{2+}-free Krebs' + EGTA
0.12 ± 0.08 (18)	0.15 ± 0.20 (7)	3.2 ± 1.3 (18)	1.2 ± 1.1 (12)	2.2 ± 1.7 (6)	0.37 ± 0.55 (7)

Table 1 Synovial vascular permeability (Mean ± S.D.). Effect of indomethacin (7×10^{-5}M) or Ca^{2+}-lack on histamine (2.5×10^{-4}M)-induced increase in ^{125}I-albumin leakage into synovial perfusate. Calculated as 100 x (perfusate count. $sec.^{-1}ml^{-1}$)/ (blood count.$sec.^{-1}ml^{-1}$). Group size in parentheses.

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Al-Haboubi, H.A. & Zeitlin, I.J. (1980). In Inflammation: Mechanisms and Treatment. Ed. Willoughby, D.A. and Giroud, J.P. pp.237-240.
Famaey, J.-P. et al (1975). Seminars in Arthritis and Rheumatism, 5, 63-81.
Northover, A.M. (1975). Br. J. Exp. Path. 56, 52-61.

THE EFFECTS OF PRIMYCIN AT THE SNAKE NEUROMUSCULAR JUNCTION

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Primycin, an antibiotic active against gram positive bacteria, depolarizes frog skeletal muscle (Kövér et al, 1976) and increases resting, but not evoked, acetylcholine (ACh) release in the guinea pig ileum and rat brain slices (Adam-Vizi et al, 1980). Its effect on ACh release has been attributed to a depolarizing action on nerve terminals resulting in spontaneous firing, which can be blocked by tetrodotoxin (TTX). Primycin has also been shown to increase the calcium permeability of the mitochondrial inner membrane (Mészáros et al, 1980). The effects of primycin have now been examined on neuromuscular transmission in the costocutaneous nerve-muscle preparation of the garter snake.

Primycin was dissolved in snake physiological solution containing 0.5% dimethylsulphoxide (DMSO) and all control measurements were made in this solution. In concentrations above 2×10^{-7}M, primycin produced a concentration-dependent reduction in resting membrane potential in the twitch muscle fibres. Concentrations of 10^{-7}M and above produced a dramatic increase in miniature endplate potential (mepp) frequency that was both time- and concentration-dependent and that faded with prolonged exposure to the drug. This effect was seen both in the presence and absence of TTX (10^{-7}M). Primycin (3×10^{-7}M) increased mepp frequency from 0.55 ± 0.18 s^{-1} to a maximum of 181.5 ± 49.9 s^{-1}, in 36 ± 5 min ($n = 4$). In Ca^{2+}-free solutions containing 0.5 mM EGTA, primycin (3×10^{-7}M) increased mepp frequency from 2.27 ± 1.9 s^{-1} to a maximum of 187.8 ± 42.5 s^{-1}, but this occurred more slowly, in 64 ± 17 min ($n = 4$). The increases in frequency in the presence and absence of extracellular calcium were not significantly different ($P > 0.05$). The increase in mepp frequency due to 20 mM K^+ was prevented in preparations bathed in Ca^{2+}-free, EGTA-containing solutions. It is concluded that the increase in spontaneous ACh release produced by primycin may be partially due to nerve terminal depolarization and partially to the release of Ca^{2+} from intraterminal mitochondria. In concentrations greater than 2×10^{-7}M, primycin produced a marked time-dependent reduction in quantal content in both Mg^{2+} paralysed and cut muscle preparations. In voltage clamped cut muscle fibres, endplate current (epc) quantal content decreased from control levels of 149 ± 22 ($n = 10$) to one or two quanta. No reduction in quantal size was seen. In voltage clamped fibres, primycin (2×10^{-7}M) produced a time-, but non-voltage-dependent prolongation of the time constant (τ) of decay of epcs. As DMSO (0.5%) alone produced a similar prolongation of τ, it was concluded that this apparent effect of primycin on τ could be attributed to the solvent.

These studies confirm that primycin is an extremely potent antibiotic in promoting resting ACh release. However, in contrast to other sites, at the neuromuscular junction in skeletal muscles its action is not solely a consequence of depolarization.

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Adam-Vizi, V. et al (1980) Pharmacology 20, 268 .
Kövér, A. et al (1976) Studia biophys. Berlin 56, 37.
Mészáros, L. et al (1980) J. Antibiotics 33, 523.

PROPERTIES OF ACETYLCHOLINE RECEPTORS ON MYOTUBES CULTURED FROM THYMUS AND SKELETAL MUSCLE OF NEONATAL RATS

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The high incidence of thymus abnormalities and the therapeutic value of thymectomy in myasthenia gravis suggest a role for the thymus in the pathogenesis of this disease. The presence of antibodies directed against the acetylcholine receptor of skeletal muscle in the serum of myasthenic patients indicates that myasthenia gravis is an autoimmune disease. Demonstration of myoid cells bearing acetylcholine receptors in the thymus led to the hypothesis that the initial antigenic stimulus could be provided by these thymic acetylcholine receptors (Kao & Drachman, 1977; Wekerle & Ketelsen, 1977). To test for differences between normal and thymic acetylcholine receptors, myotubes were grown in cell cultures derived from thymus and from skeletal muscle of 1-4 day old rats and the properties of the acetylcholine receptors were compared using conventional intracellular recording techniques.

In both types of culture, multinucleated fibres were formed by fusion of uninucleated myoblasts, although fusion took place earlier in skeletal muscle cultures. Myoblasts from skeletal muscle fused on day 3 in culture whereas thymus cells differentiated into phase-refractile spindle-shaped cells on day 4 which fused on day 6. There was no significant difference between resting membrane potentials in thymus and skeletal muscle cultures. Resting potentials of myoblasts and small myotubes in both thymus and muscle cultures were -11±0.6 mV (mean±s.e. mean of 30 values from 8 cultures). In both types of cultures the resting potential increased with age. Two days after fusion the mean membrane potential had increased to -51±0.8 mV (mean±s.e. mean of 40 values from 8 cultures) and this level was approximately constant throughout the period of culture (4-5 weeks).

In both types of culture, small myotubes just after fusion were relatively insensitive to ionophoretic application of acetylcholine. In the first 3 days after fusion high sensitivity to acetylcholine appeared in all cells, which were capable of being depolarized to about 0 mV. The average sensitivity to acetylcholine was the same in both types of cultures, and there was a four-fold decrease in the average sensitivity with age in culture.

The acetylcholine receptors of myotubes cultured from skeletal muscle and thymus both appeared to be nicotinic in properties. Tubocurarine (1-2 μM) and the snake venom toxin erabutoxin b (20 nM) antagonised the responses to acetylcholine. Tubocurarine behaved as a competitive antagonist and had similar affinities for the acetylcholine receptor in both types of culture: the pA_2 values for tubocurarine against acetylcholine were 6.7±0.3 (n=4) for myotubes cultured from thymus and 6.2±0.1 (n=4) for myotubes cultured from skeletal muscle. In contrast, the muscarinic receptor antagonist atropine was relatively ineffective in blocking responses to acetylcholine, having a pA_2 of about 4.5.

From the present results, the morphological and electrophysiological development of myotubes in cultures derived from neonatal rat thymus appears to be similar to that of myotubes in skeletal muscle cultures, and there do not appear to be major differences in the pharmacological selectivity of thymus and skeletal muscle acetylcholine receptors.

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Kao, I. & Drachman, D.B. (1977) Science 195, 74.
Wekerle, H. & Ketelsen, U.P. (1977) Lancet i, 678.

THE RELEASE OF A FALSE TRANSMITTER FROM THE RAT PHRENIC NERVE-DIAPHRAGM

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A number of analogues of choline have been shown to be incorporated into cholinergic nerve terminals (Collier, et al, 1979). The tri-ethyl analogue of choline (TEC) has been shown to be effective at the neuromuscular junction (Bowman et al, 1962) and the compound has been shown to be taken up and acetylated in vivo by the cat superior cervical ganglion (Ilson, et al, 1977). The present experiments investigated the incorporation of TEC at the neuromuscular junction of the rat - phrenic nerve diaphragm preparation and determined the amount of acetyl-TEC formed. A method of gas chromatography/mass spectrometry was used to analyse the false transmitter produced: this method has not been previously used for determination of false transmitter activity. The diaphragm preparation has been utilized on a number of occasions for investigation of the storage and release of acetylcholine (ACh) (Bowman & Hemsworth, 1967, Potter 1970).

The hemidiaphragm was mounted in an organ bath of volume 3 mls and suspended in 2.5 mls McEwens (1956) physiological saline solution. Physostigmine 5 µg/ml was added to the preparation to inhibit acetylcholinesterase and the phrenic nerve was stimulated at a frequency of 1.0 Hz.

To determine the neurotransmitter released as a result of nerve stimulation the solution bathing the diaphragm was collected, and an appropriate amount of butyrylcholine and butyryl-TEC was added to the solution to act as internal standards.

The quantity of neurotransmitter released into the bathing solution was estimated by pyrolysis gas chromatography and the identity of the neurotransmitter was confirmed by means of mass spectrometry.

In the control diaphragms 11.0 pmoles of ACh was released from the diaphragm in a 15 min. period. After a period of stimulation of 4 hours, in the presence of TEC 500 µg/ml, the amount of ACh released within the 15 min period was reduced to 5.7 pmoles. At the same time 33 pmoles acetyl-TEC was also released from the rat diaphragm.

The results confirmed the release of acetyl-TEC as a false transmitter from the phrenic nerve endings when the parent analogue TEC is added to the bathing fluid.

Bowman, W.C. & Hemsworth, B.A. (1965). Br. J. Pharmac.Chemother. 25:392.
Bowman, W.C. et al (1962). Br.J.Pharmac.Chemother. 19:198.
Collier, B. et al (1979). Prog. Brain Res. 49:107.
Ilson, D. et al (1977). J. Neurochem. 28:371.
Potter, L.T. (1970). J. Physiol. Lond. 206:145.

THE BINDING OF (^{3}H)-PRAZOSIN AND (^{3}H)-CLONIDINE TO CRUDE BASO-LATERAL MEMBRANES FROM RAT JEJUNUM

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Noradrenaline stimulates sodium and water absorption from a variety of intestinal preparations (Levens et al, 1979). This response is inhibited by α but not β adrenoceptor antagonists. It would be anticipated therefore, that intestinal epithelial would possess α adrenoceptors. The present study has examined this possibility using [^{3}H]-prazosin and [^{3}H]-clonidine.

Epithelial cells were isolated from rat jejunum and crude baso-lateral membranes prepared from these cells by the methods of Murer et al (1973) and Scalera et al (1980). The activity of Na K ATP'ase (a marker for baso - lateral membranes) was increased from 0.287 to 1.176 µmol Pi per min per mg protein (cells to membranes). Similarly, alkaline phosphatase (a marker for brush border membranes) was decreased from 2.54 (cells) to 1.8 µmol paranitrophenylphosphate hydrolysed per min per mg protein (membranes). This suggests that a partial enrichment of baso-lateral membranes had occurred. Binding was carried out in lysed membranes (300-500 mg protein per ml) in the presence of 2 nM [^{3}H]-prazosin or 15 nM [^{3}H]- clonidine (S.A. 28 and 20 Ci per mMol respectively). The buffer was 50 mM tris HCl, pH 7.6 and specific binding was defined by 1 µM prazosin or 10 µM clonidine. After incubation for 10-16 min at 25 °C free and bound ligand were separated by filtration (Whatman GF/B filters) and assayed for radioactivity. Under these conditions specific [^{3}H]-prazosin binding was 40-50% specific [^{3}H]-clonidine 50-80% of the total binding.

Specific binding of both ligands was saturable, in both cases Scatchard analysis revealed two binding sites (ligand concentration 0-40 nM [^{3}H]-prazosin, 0-40 nM [^{3}H]-clonidine). The characteristics of the high affinity sites was Kd 6.65 nM, Bmax 11.2 fmol per mg protein; Kd 0.94 nM Bmax 33 fmol per mg for [^{3}H]-clonidine and [^{3}H]-prazosin respectivley. In separate experiments, the equilibrium dissociation constant for [^{3}H]-prazosin binding was determined from K_1 (association) and K_2 (dissociation) constants and gave a value of 2.5 nM which is in agreement with the value determined from saturation analysis. The ability of a series of α_1 and α_2 agonists and antagonists to displace specific ligand binding was determined and the values expressed as Ki values. The rank order of potency for [^{3}H]-prazosin binding was prazosin (3.0 nM); phentolamine (305 nM); WB4101 (437 nM); clonidine (6.4 µM); yohimbine (8.6 µM) and phenylephrine (352 µM) and for [^{3}H]-clonidine binding the order of potency was clonidine > noradrenaline > phenylephrine > phenoxybenzamine.

These preliminary observations suggest that basolateral membranes from epithelial cells possess a mixed population of α adrenoceptor binding sites, and are in agreement with those of Tanaka and Starke (1979) who found [^{3}H]-clonidine binding sites in membranes from guinea-pig ileum.

Levens, N.R. et al (1979) J. Physiol. 286, 351-360.
Murer, H. et al (1974) Biochem. Biophys. Acta. 345, 170-179.
Scalera, V. et al (1980) Biochem. J. 186, 177-181.
Tanaka, T. and Starke, K. (1979) Naunyn-Schmeideberg Arch. Pharmacol. 309, 207-215.

INHIBITORY RESPONSES TO VIP AND TO ATP IN THE ISOLATED ANOCOCCYGEUS MUSCLE OF THE MOUSE

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The isolated anococcygeus muscle of the mouse has been shown to relax in response to vasoactive intestinal polypeptide (VIP) and, following incubation with indomethacin, to adenosine triphosphate (ATP, Gibson & Wedmore, 1981). Both of these substances have been proposed as possible neurotransmitters of non-adrenergic, non-cholinergic nerves to the pelvic viscera. Since the anococcygeus muscles receive such an inhibitory innervation we have investigated more closely the properties of the inhibitory responses to VIP and ATP in the isolated anococcygeus muscle of the mouse.

Male mice (25-35g) were killed by stunning and exsanguination, and the anococcygeus muscles dissected and set up in organ baths as described previously (Gibson & Wedmore, 1981). In order to reveal inhibitory responses in this normally quiescent tissue tone was raised by carbachol (10-50μM), the motor responses having been negated by guanethidine (30μM) or phentolamine (1μM).

VIP (10nM-5μM) produced dose-related inhibitions of carbachol-induced tone in the mouse anococcygeus muscle. In other experiments VIP also relaxed the isolated anococcygeus muscles of the rabbit and rat. Following incubation with indomethacin (2.8μM;1 hour), ATP (500μM-10mM) also produced dose-related relaxations of the mouse anococcygeus muscle. The inhibitory responses to field stimulation (10Hz; 10s), VIP, and ATP were unaffected by apamin (500nM) which blocks ATP-induced relaxations of the guinea pig taenia caeci (MacKenzie & Burnstock, 1980). The proposed ATP antagonist 2-2'-pyridylisatogen tosylate (50μM) by itself markedly reduced carbachol-induced tone but, even so, in its presence field stimulation, VIP, and ATP could still elicit muscle relaxation. Haemolysed blood, which reduces inhibitory nerve responses in the bovine retractor penis (Bowman & Gillespie, 1981), had a similar effect in the mouse anococcygeus muscle but it did not affect responses to VIP or ATP. During prolonged inhibitory field stimulation (10Hz;10-20min) muscle tone often returned towards, and in some cases reached, the prestimulation level. Addition of VIP at this time failed to cause muscle relaxation although ATP produced normal inhibitions.

The results suggest that the mechanisms underlying inhibitory responses of the mouse anococcygeus muscle to field stimulation and ATP are similar to those of the bovine retractor penis and unlike those of the guinea pig taenia caeci. The selective desensitisation of VIP-induced relaxations during prolonged inhibitory nerve stimulation is being investigated.

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Bowman, Anne & Gillespie, J.S. (1981) J. Physiol. 317, 92P-93P
Gibson, A. & Wedmore, C.V. (1981) J. Auton. Pharmac. 1, 225-233
MacKenzie, I. & Burnstock, G. (1980) Eur. J. Pharmac. 67, 255-264

THE EFFECT OF PRIOR TREATMENT WITH 4-DIMETHYLAMINOPHENOL (DMAP) IN EXPERIMENTAL HYDROGEN CYANIDE POISONING

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4-Dimethylaminophenol (DMAP) is a substance which has been investigated for use in the treatment of cyanide poisoning (Kiese and Weger, 1969). Although the therapeutic action of DMAP is similar in mechanism to that of sodium nitrite the former drug has the advantage that in man it has little effect on the circulation (Daunderer, 1979). Dogs were used for the experiment because of the similarity of their methaemoglobin reductase system to that in man. Cyanide was estimated by a spectrophotometric method (Epstein, 1947) which was preceded in the case of the blood samples by a microdiffusion procedure using a Conway dish (Feldstein & Klendshoj, 1954). Methaemoglobin was measured using an IL 282 Co-oximeter.

Preliminary experiments were carried out in each dog, to establish the dose of DMAP which, when administered with sodium bicarbonate, by gavage or capsule, would produce a peak methaemoglobinaemia of 12-15%. The cephalic vein of one fore-limb was cannulated and a control blood sample taken for methaemoglobin estimation. On 3 occasions the required dose of DMAP dissolved in 5 ml distilled water was administered by gavage immediately followed by 10 ml of a 0.5 g ml^{-1} slurry of sodium bicarbonate. On a further 3 occasions the DMAP was given in a gelatine capsule with 1 g sodium bicarbonate. Following both methods of administration blood samples for methaemoglobin were taken at 8 min and thereafter. They were estimated immediately and when the level reached 8-10%, 2.7 mg kg^{-1} hydrogen cyanide, diluted with water so that the dose was present in 2 ml of solution, was injected i.v. over 20 sec. The animal was observed until death or complete recovery took place. On 3 further occasions dogs were given hydrogen cyanide i.v. as described above, but without protection with DMAP. All the protected animals had marked signs of cyanide intoxication which started at the end of the injection of hydrogen cyanide, but full recovery occurred within one hour in four out of six animals. One dog appeared to recover well but had two grand-mal epileptiform seizures at 210 min, which were terminated by injection of sodium thiosulphate. The sixth dog died 44 min after injection of the cyanide. Measurement of cyanide levels showed that the plasma levels were very much below the whole blood levels, the ratio being 0.076 and 0.0165 for the dogs given DMAP by gavage and by capsule respectively.

In the 3 unprotected animals signs of cyanide poisoning started at the cessation of injection of the hydrogen cyanide. Respiration and pulse ceased within 1½ min and the corneal reflex was lost. The mean plasma cyanide in these 3 animals was higher than the mean whole blood cyanide, the ratio between the two being 1.27. It has been suggested that plasma cyanide is the determinant of tissue levels and toxic effects of cyanides (Vesey, 1979; Ballantyne, 1979) and if this were so the difference in plasma: whole blood cyanide ratios in the protected and unprotected dogs would explain the efficacy of DMAP.

Ballantyne, B. (1979). Letter. Clin Toxicol, 14, 311.
Daunderer, M. (1979). Dissertation for the appointment as Reader. University of Munich FRG.
Epstein, J. (1947). Analytical chem, 19, 272.
Feldstein, M & Klendshoj, N.C. (1954). J Lab Clin Med, 44, 166.
Kiese, M. & Weger, N. (1969). Europ J Pharmacol, 7, 97.
Vesey, C.J. (1979). Letter. Clin Toxicol, 14, 307.

CLOFIBRATE INDUCES AN UNUSUAL FORM OF HEPATIC CYTOCHROME P-450 RESPONSIBLE FOR ALTERED DRUG AND FATTY ACID METABOLISM

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Clofibrate (a widely-used hypolipidemic drug) is well known to produce proliferation of both peroxisomes and the endoplasmic reticulum (microsomes) of rodent liver (Hess et al., 1965; Azarnoff et al., 1975). Although the clofibrate-associated morphological changes in liver have been extensively investigated, less information is available regarding the drug-metabolising activity of clofibrate-induced liver microsomes. Accordingly, we now report the influence of clofibrate pretreatment on the drug and fatty acid oxidising capabilities of rodent liver microsomes.

Male Wistar rats (180-200g) were given i.p. injections of clofibrate (50-400mg/kg) once daily for three days, the animals killed on the fourth day and liver microsomal fractions prepared by differential ultracentrifugation. A dose-dependent increase in the microsomal cytochrome P-450 specific content (nmol/mg protein) was observed, reaching 160% of controls at the higher dose levels. In addition, the clofibrate-induced cytochrome P-450 exhibited an altered catalytic activity towards the oxidation of benzphetamine and aminopyrine, as well as the medium chain saturated fatty acid, lauric acid (Table 1).

TABLE 1. Influence of clofibrate pretreatment on rat liver microsomal oxidations.

Substrate	control microsomes	clofibrate-induced microsomes
Benzphetamine[a]	12.00 ± 0.10	4.90 ± 0.09
Aminopyrine[a]	11.87 ± 1.26	5.93 ± 0.10
Lauric acid[b]	1.53 ± 0.25	7.55 ± 0.20

Metabolism is expressed as either [a] nmol HCHO formed/nmol cytochrome P-450/min or [b]total 11-plus 12-hydroxylauric acid formed/mg protein/min (Mean ± S.E.M. for 4-6 determinations).

The above induction of cytochrome P-450 by clofibrate was partially blocked by the co-administration of actinomycin D, indicating that de novo protein synthesis was required in the induction process. Thus our results indicate that clofibrate induces a unique form of cytochrome P-450 that preferentially metabolises fatty acids. To further substantiate this hypothesis, the clofibrate-induced cytochrome P-450 was purified by chromatographic techniques (Guengerich, 1978) and the properties of this cytochrome P-450 compared to that purified haemoprotein induced by phenobarbital pretreatment (i.e. the cytochrome P-450 isoenzyme that actively metabolises various drugs). An analysis of these two haemoproteins revealed significant difference based on spectral properties, monomeric molecular weights and catalytic activities with respect to the oxidation of benzphetamine and lauric acid.

Although these results do not clearly delineate the role of cytochrome P-450 in the mode of action of clofibrate, they point to possible drug-drug interactions involving clofibrate therapy.

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Azarnoff, D.L. et al. (1975) Proc. Sixth Int. Congr. Pharmacol. 4, 137.
Hess, R. et al. (1965) Nature 208, 856
Guengerich, F.P. (1978) J. Biol. Chem. 253, 7931.

TISSUE CONCENTRATIONS OF PROPRANOLOL IN RATS WITH ADJUVANT-INDUCED ARTHRITIS

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It has been shown previously that following single oral and i.v. doses, plasma concentrations of propranolol are significantly elevated in rats with adjuvant-induced arthritis (Bishop et al, 1981). Since increased plasma concentrations may well result in a shift in the distribution of the drug in these animals, the work has been extended by measuring propranolol in lung, brain, heart and liver under similar conditions.

Propranolol was given orally (2mg) and i.v. (0.25mg) to normal rats, and to rats with adjuvant-induced arthritis as previously described (Bishop et al, 1981). These doses have been found to give similar peak plasma levels in normal animals. At various time-intervals after dosing, the rats were anaesthetised and bled from the aorta into 3.8% sodium citrate solution. Lungs, brains, hearts and livers were quickly removed, blotted, weighed and homogenised with Tris/HCl buffer (0.05M, pH 7.4) to give a final concentration of 1g (wet weight) in 10ml suspension. The homogenates, together with the separated plasma, were stored at -20°C, until propranolol was measured by the fluorometric method of Shand et al (1970).

After oral administration, plasma and tissue concentrations were raised in the arthritic rats by, for example, as much as 16-fold for plasma and between 2 and 3-fold in tissues at 30 min (peak plasma levels). Elevated concentrations were observed for up to 1h after dosing.

In contrast, after i.v. administration, although plasma concentrations were also significantly raised above control values at the first two sampling times (10 min, 20 min), the levels in lung, brain and heart were significantly lower, and remained so for the duration of observations.

Although there was some variation in actual plasma and tissue levels, particularly after oral administration, the individual tissue/plasma ratios were remarkably consistent for rats within the same group. These ratios were much smaller in the arthritic than in the normal rats after both oral and i.v. dosing. For instance, in lung, brain and heart, 20 min after an i.v. dose, the ratios in normal rats were 134, 25 and 11 respectively, and in arthritic animals 34, 7 and 2.5. These figures would suggest that in the arthritic rats, the volume of distribution was altered and a smaller proportion of the body-load was being distributed to the tissues. Interestingly, in spite of this change, tissue levels in the arthritic rats after oral administration were actually raised compared with the normals. This supports the hypothesis that there is decreased first-pass metabolism in these animals and thus a greater amount of unmetabolised drug is available to produce an increased pharmacological effect in the tissues.

Bishop, H., Schneider, R.E. & Welling, P.G. (1981) Biopharm.Drug Disp. 2, 291-297.
Shand, D.G., Nuckolls, E.M. & Oates, J.A. (1970) Clin.Pharmacol.Ther. 11, 112-120.

EFFECT OF TAMOXIFEN METABOLITES ON PEROXIDASE ACTIVITY IN THE RAT UTERUS

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Tamoxifen, an antiestrogen, and its metabolite 4 hydroxytamoxifen (HT) can exert estrogenic effects on the rat uterus (Jordan & Dix, 1979). The abilities of the tamoxifen metabolites HT, dihydroxytamoxifen (DOT) and N-desmethyl-tamoxifen (NDES) to induce peroxidase activity in the rat uterus have been compared as an index of estrogenic potency (Jellinck & Newcombe, 1977). Rats 19-21 days old were primed with 5 μg estradiol followed, 5 days later by the test dose given s.c. in arachis oil. 18 h after this, peroxidase activity was measured in the high-salt (1.2 M NaCl or 0.5 M $CaCl_2$) extract of the particulate material of the uterus, using guaiacol as substrate (Jellinck & Newcombe, 1977).

The results in Figure 1 confirm the ability of HT to increase uterine peroxidase activity (and uterine weight). NDES resembles tamoxifen itself in inducing a modest increase in uterine weight (especially at 100 μg/rat) but not in peroxidase activity. DOT causes little increase in either parameter, nor does it affect the increases brought about by estradiol (1 μg) or HT (5 μg). Peroxidase induction by 5 μg HT (but not the uterine weight gain) is inhibited by concurrent injection of 500 μg progesterone and thus resembles that due to estradiol (Anderson et al 1977).

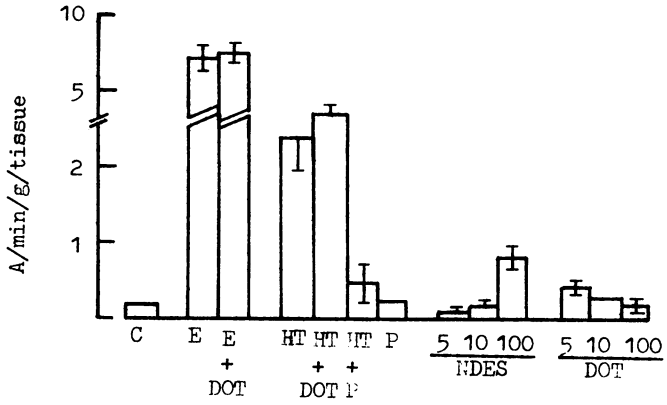

Figure 1 Peroxidase activity (ΔA_{470nm}/min/g tissue) in uteri of estrogen-primed immature rats 18 h after a single s.c. injection of vehicle (C), 1 μg estradiol (E), 500 μg progesterone (P), 5 μg hydroxytamoxifen, (HT), 100 μg dihydroxytamoxifen (DOT), or 100 μg N-Desmethyltamoxifen (NDES). Effects of simultaneous administration are also shown. Where differing amounts were given the dose in μg is indicated under the relevant bar. Results are expressed as mean values (± S.E.M. for n = 4 - 11 experiments).

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Anderson, W.A. et al (1977) Biol. Reprod. 16, 409.
Jellinck, P.H. & Newcombe, A. (1977) J. steroid Biochem. 8, 1193.
Jordan, V.C. & Dix, C.J. (1979) J. steroid Biochem. 11, 285

THE USE OF ACID AND HYDROLASES FOR ANALYSING BILIARY CONJUGATES OF OESTROGENS

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During a study of the biliary conjugates of 17α-ethynyloestradiol (EE_2) and its metabolites in the rat, we have examined several commercial enzyme preparations in respect of the degree and selectivity of hydrolysis. They were categorised as arylsulphatases, β-glucuronidases and mixed preparations on the basis of their ability to hydrolyse ^{3}H-oestrone glucuronide (E_1G) and ^{3}H-oestrone sulphate (E_1S). Hydrolysis by β-glucosidase was investigated because a glucose-containing diglycoside of oestradiol is formed in the rabbit.

Biliary metabolites of ^{3}H-EE_2 were obtained from male rats and analysed by h.p.l.c. (Breckenridge et al., 1981). Bile was incubated with hydrolases under optimal conditions, and the deconjugated ^{3}H-metabolites extracted into ether prior to h.p.l.c. analysis. The results are given as $\bar{x}$ (n=4) ± s.d.

Table 1 Enzymic Hydrolysis of Biliary Metabolites of EE_2 and E_1 Conjugates

Enzyme Preparations	% ^{3}H extracted into ether			Deconjugated ^{3}H metabolites			
	E_1G	E_1S	Rat Bile	2-OHEE$_2$	EE_2	2-MeOEE$_2$	2-OHM
H-1 (H.pomatia)	97 ± 0	99 ± 1	61 ± 6	13 ± 2	14 ± 1	29 ± 6	6 ± 1
H-2 (H.pomatia)	97 ± 0	99 ± 0	72 ± 4				
Abalone Arylsulphatase	5 ± 1	98 ± 1	28 ± 8	6 ± 2	8 ± 3	41 ± 8	3 ± 1
Beef liver β-Glucuronidase	98 ± 1	4 ± 1	46 ± 3	8 ± 3	23 ± 4	27 ± 5	8 ± 1
E.coli β-Glucuronidase	97 ± 0	1 ± 1	41 ± 6	5 ± 1	27 ± 2	33 ± 3	10 ± 1
β-Glycosidase	41 ± 2	65 ± 1	42 ± 10	13 ± 2	12 ± 3	39 ± 6	4 ± 3

The mixed preparations from H.pomatia effected the greatest hydrolysis of biliary metabolites. The arylsulphate ester fraction was estimated by employing the preparation from abalone; Aerobacter aerogenes arylsulphatase acted too slowly (11 ± 2% (n=3) ^{3}H extracted into ether after 6h) to be of practical use. Microbial and mammalian β-glucuronidases liberated equal quantities of aglycones. H.p.l.c. analysis showed that EE_2 and its principal metabolites: 2-hydroxyethynyloestradiol (2-OHEE$_2$), 2-methoxyethynyloestradiol (2-MeOEE$_2$) and 2-hydroxymestranol (2-OHM) (Brown et al., 1981), formed both sulphate esters and glucuronides. β-Glucosidase catalysed extensive hydrolysis of ^{3}H-EE_2 biliary metabolites, but was found to possess arylsulphatase and β-glucuronidase activity.

Following ether extraction and re-incubation, extraction of ^{3}H from H-2 incubations rose to 82 ± 1% (n=3). Although 72% of biliary ^{3}H was extracted into ether after refluxing with 2M HCl for 2h, this decreased on longer refluxing. Since it was suspected that the biliary metabolites of ^{3}H-EE_2 insusceptible to hydrolysis might be amino acid conjugates, ^{35}S-cysteine and EE_2 were administered together. However, excepting a minor component also found in bile from rats given ^{35}S-cysteine alone, none of the ^{35}S-components co-chromatographed with ^{3}H-EE_2 metabolites.

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Breckenridge, A.M. et al., (1981) Oxford Meeting, September.

Brown, I.C. et al., (1981) Bradford Meeting, April.