QUININE INHIBITS CONTRACTILITY OF RAT ANOCOCCYGEUS MUSCLE.

A. O. Savage, Department of Pharmacology & Therapeutics, College of Medicine, University of Ibadan, Ibadan.

Quinine exerts prominent sympatholytic action on the cardiovascular system (Hiatt, 1950). It also blocks muscarinic cholinoceptors on the guinea-pig myocardium (Mirro et al., 1980) and the rat rectum (Savage & Akinlalu, 1985). Other recent work suggests that quinine blocks CNS α-adrenoceptors (de Zoeten et al., 1983). The present study examined the effects of quinine on the sympathetically-innervated rat anococcygeus muscle, in an attempt to elucidate the mechanism of its sympatholytic action.

Anococcygeus muscles were removed from male Wistar rats and set up for isometric recording (Gillespie, 1972). Responses to electrical field stimulation (EFS), as well as to a range of concentrations of noradrenaline (NA), methoxamine (ME), clonidine (CLO), carbachol (CCh), KCL and $CaCl_2$ in depolarising (120mM K^+) Tyrode solution were determined first in the absence then in the presence of varying concentrations of quinine. The influence of quinine of NA and CCh-induced contractions evoked in Ca^{2+}-free Tyrode (CFT) was also studied.

Quinine concentration-dependently blocked both motor and inhibitory responses evoked by EFS (IC_{50} 5 μM and 15 μM, respectively). Quinine non-competitively antagonised agonist-induced contractions. Quinine IC_{50} values were: $CaCl_2$ 2.5 nM, ME 0.36 μM, KCL 17 μM, NA 50 μM, CCh 50 μM, CLO 77 μM. The quinine IC_{50} of $CaCl_2$-induced responses (2.5 nM), was at least 2,000 times less than the quinine IC_{50} value against sympathetic nerve-induced contractions (5 μM), and 20,000 times less than the quinine IC_{50} against NA (50 μM). Quinine also blocked NA and CCh contractions in CFT with about the same potency as was obtained in normal Tyrode, suggesting that this quinine effect occurred by an intracellular action. It may be concluded that quinine possesses potent "Ca^{2+} antagonistic" activity, as well as antiadrenergic and antimuscarinic actions. Quinine blockade of nerve-induced responses may be attributable to quinine blockade of nerve-terminal voltage-dependent Ca^{2+} channels, which would reduce neurotransmitter output, while higher concentrations inhibit muscle contractility by a direct intracellular action exerted on the smooth muscle fibres.

Supported by University of Ibadan Senate Research Funds.

Gillespie, J.S. (1972). Br. J. Pharmac. 45, 404-416.
Hiatt, E.P. (1950). Am. J. Physiol. 160, 212-216.
Mirro, M.J., Manalan, A.S., Bailey, J.C. & Watanabe, A.M. (1980). Circ. Res. 47, 855-856.
Savage, A.O. & Akinlalu, C.A. (1985). Arch. Int. Pharmacodyn. Ther. 276, 163-176.
de Zoeten, K., van Meel. J.C.A., van Kemenade, J.E., Batink, H.D., Timmermans, P.B.M.W.M. & van Zweiten, P.A. (1983). Arzneim. Forsch. 33, 694-698.

DIRECT EXCITATORY AND INHIBITORY EFFECTS OF TWO BENZODIAZEPINES ON NEUROMUSCULAR TRANSMISSION.

R.S. Leeuwin, H. van Wilgenburg, B.F.M. Werdmuller and A. Zeegers, Department of Pharmacology, University of Amsterdam, Academic Medical Centre, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Benzodiazepines have muscle relaxant properties, which are generally thought to be of central origin. However, some of these effects may nevertheless be due to direct peripheral actions at the neuromuscular junction, although reports poing in this direction are rather controversial. A minireview of the current state of affairs has recently been published by Wali (1985). Effects of diazepam on the neuromusculair transmission in in vitro nerve-muscle preparations have been described as absent (Crankshaw and Raper, 1968), potentiating (Moodgil and Pleuvry, 1970) and inhibitory (Vyskocil, 1977, 1978). A biphasic action of diazepam on the phrenic nerve-diaphragm preparation has been described by Driessen et al. (1984).

Direct effects of benzodiazepines at the neuromuscular junction were studied 1. by investigating the effect of two related 1,4-benzodiazepines, diazepam and chlorodiazepoxide, on the twitch tension of the rat phrenic nerve-diaphragm preparation in vitro; 2. by measuring the synaptic vesicle size in nerve endings of diaphragm preparations after i.p. injection of diazepam.

In all studies female rats (Cpb WU:WI), 170-200 g were used.

The results may be summarized as follows. As a rule concentrations of diazepam up to 0.0875 mM/l have no effect on the twitch tension. Concentrations of 0.0875 to 0.2625 mM/l produce an increase of the twitch tension. The increase caused by the last dose is followed by a depression. After washing, recovery of the twitch tension usually is 95-110%. In general no effect on the base line is observed.

Chlorodiazepoxide was administered in concentrations ranging from 0.0875-0.50 mM/l. No increase of the twitch tension was noted. Depression takes place only at the relatively high concentrations of 0.50 mM/l.

Electron microscopic studies of the endplate rich areas of rat diaphragms have shown that the cross sectional area of the synaptic vesicle profiles is increased significantly ($P<0.005$) by diazepam in doses of 1.0, 2.5, 5.0 and 10 mg/kg. The increase is directly proportional to the treatment interval (15, 30, 60 and 90 min) and dose-related, although less pronounced at the longest treatment intervals.

It is tentatively concluded that benzodiazepines may interfere directly with events at the neuromuscular junction. The increase in vesicle size caused by diazepam may be related to modification of the twitch tension.

Crankshaw D.P. and C. Raper, Br.J.Pharmac. (1968) 34, 579-590.
Driessen J.J., T.B. Vree, L.H.D.J. Booij, F.M. van der Pol and J.F. Crul, J.Pharmacol. (1984), 35, 244-247.
Moodgil G. and B.J. Pleuvry, Br.Med.Journal (1970) 2, 734-735.
Vyskocil F., Brain Research (1977) 133, 315-328.
Vyskocil F., European J.Pharmacology (1978) 48, 117-124.
Wali F.A., Life Sciences (1985), 37, 1559-1561.

IDENTIFICATION OF α_1 ADRENOCEPTORS IN CHICK EXPANSOR SECUNDARIORUM MUSCLE.

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

The expansor secundariorum (ESM) is a discrete smooth muscle in the wing of the chick. Catecholamines cause contraction of the ESM in vitro, an effect mediated by α- rather than by β-adrenoceptors (Buckley and Wheater, 1968). We have now identified α_1-adrenoceptors in ESM using ligand binding and organ-bath techniques.

1. Ligand binding studies

Chicks (25 to 50 day old) were killed by decapitation, the ESMs removed and homogenized in HEPES-buffered physiological medium (composition mM: NaCl 110, KCl 5.3, $CaCl_2$ 11.8, $MgSO_4$ 0.8, NaH_2PO_4 0.9, glucose 25, sucrose 50 and HEPES 20, pH 7.4). Aliquots (400-600 µg protein) of ESM homogenate were incubated with the α_1-adrenoceptor selective ligand [^{3}H]-prazosin at 25°C, usually for 30 minutes and non-specific binding was defined with 3µM phentolamine. Saturation binding analyses were performed to calculate the number and affinity of [^{3}H]-prazosin binding sites in ESM. To study the affinity of various adrenoceptor-selective ligands for this site, ESM homogenate was incubated with [^{3}H]-prazosin and increasing concentrations of competing ligand. Association and dissociation of [^{3}H]-prazosin binding were studied using standard techniques. All assays were performed in triplicate or quadruplicate and terminated by filtration over Whatman GF/B filters.

Specific binding of [^{3}H]-prazosin was saturable, rapid ($k_{ass} = 3 \times 10^8$ M^{-1} min^{-1}) and reversible ($k_{diss} = 0.26$ min^{-1}). Saturation binding analysis revealed a maximum receptor number of 24.4 fmoles/mg protein and an affinity (K_D) of 0.76nM. In displacement studies, adrenoceptor antagonists showed an order of potency consistent with binding of [^{3}H]-prazosin to α_1-adrenoceptors; K_i values (nM) were: WB4101 0.8, phentolamine 3.8, rauwolscine 280, idazoxan 721 and propranolol 3900.

2. Organ bath studies

The ESMs were set up in 20ml organ baths and bathed with Krebs-bicarbonate buffer as previously described (Bennett et al., 1982). Noradrenaline and methoxamine (10^{-7} to 10^{-4}M) caused contraction of the ESM, both being full and potent agonists. In contrast, the α_2-adrenoceptor-selective agonist B-HT920 was virtually without effect, producing only small contractions at high (>100 µM) concentrations. The response to methoxamine was antagonised by prazosin far more potently than by rauwolscine.

We propose that the α_1-adrenoceptors labelled by [^{3}H]-prazosin in ESM are also responsible for catecholamine-mediated contraction of the muscle.

Supported by the M.R.C.

Bennett, T. et al. (1982) Br. J. Pharmac. 76, 177-183.
Buckley, G.A. and Wheater, L.E. (1968) J. Pharm. Pharmac. 20, 114S-121S.

BLOCKADE OF VASOPRESSOR AND VAS DEFERENS RESPONSES BY αβ-METHYLENE ATP IN THE PITHED RAT.

J.M. Bulloch & J.C. McGrath, Autonomic Physiology Unit,
Institute of Physiology, University of Glasgow, Glasgow G12 8QQ

We recently demonstrated that αβ-methylene ATP (mATP) could attenuate pressor responses to sympathetic nerve stimulation after blockade of alpha receptors but not in the absence of alpha-blockade (Flavahan et al, 1985; Grant et al, 1985). This seemed to suggest a relatively minor role for purinergic co-transmission in rat vasculature. We have now re-examined this taking into account the short life of mATP and demonstrate that pressor responses due to sympathetic nerve stimulation are more sensitive to mATP than was previously suggested.

Male rats (250g) were pithed under halothane anaesthesia (Gillespie et al, 1970) and were artificially ventilated with 40% oxygen and 60% nitrogen and given gallamine (10mg/kg, i.v.) to stop skeletal muscle twitching and propranolol (1mg/kg, i.v.). Drugs were administered via the right external jugular vein. Diastolic pressor responses to sympathetic nerve stimulation via the pithing rod (1cm electrode, T8, 1 sec, 5-20Hz) were monitored via the cartoid artery. Longitudinal tension responses of the vasa deferentia were recorded in situ (Gillespie et al, 1974) to sympathetic nerve stimulation (1cm electrode,T13,1sec, 5Hz).

The i.v. administration of mATP (0.01-0.5mg/kg) produced a short-lived pressor response which was subject to tachyphylaxis. Attenuation of nerve-mediated pressor responses was noted in this dose range in a time dependent manner: maximum blockade occurred 1 min after the addition of mATP but faded in 10 minutes to a maintained desensitisation. Responses to sympathetic nerve stimulation were partially blocked by the addition of alpha-adrenergic blocking agents prazosin and rauwolscine (both 1 mg/kg). In the absence of alpha-blockers, subsequent additions of mATP reduced pressor responses to sympathetic nerve stim ulation which eventually reached approximately 40% of control levels. In the presence of alpha-blockers, subsequent additions of mATP (0.5mg/kg) completely blocked the pressor responses. Under these conditions, responses of the vas deferens to sympathetic nerve stimulation were blocked. This is in contrast to the effect of alpha blockers which did not attenuate these responses of the vas deferens.

In conclusion, sympathetic vasopressor nerve transmission in the rat is mediated by both alpha-adrenergic and purinergic elements and can be partly blocked by alpha or purinergic blockers but can be completely blocked by a combination as can the responses to stimulalation of the vas deferens.

JMB holds an SERC CASE studentship in collaboration with Pfizer Central Research.

Flavahan, N.A. et al (1985) Br.J. Pharmac. 86, 265-274
Gillespie, J.S. et al (1970) Br. J. Pharmac. 40, 257-267
Gillespie, J.S. et al (1974) Br. J. Pharmac. 52, 585-590
Grant, T.L. et al (1985) Clinical Science, 68 (Suppl. 10), 25s-30s

THE EFFECT OF NEUROPEPTIDE Y (NPY) ON NEUROTRANSMISSION IN RAT VAS DEFERENS.

M.M. Iravani, V.R. Marsh & M.A. Zar, Department of Pharmacological Sciences, Medical School, Framlington Place, Newcastle upon Tyne NE2 4HH

NPY, a 36 amino acid peptide, is present in the post-ganglionic autonomic nerves of rat vas deferens (Lundberg et al. 1982). NPY has been reported to inhibit the electrically-evoked neurogenic contraction of rat vas deferens (Lundberg & Stjarne, 1984; Huidoro-Toro, 1985). It has been shown previously that the indirect mechanical response of the isolated rat vas deferens preparation to a single electrical pulse originates from the activation of different populations of nerve terminals and the contribution of each nerve population to the overall response can be evaluated by using electrical pulses ranging in pulse duration from 0.04 ms to 1.8 ms (Zar, 1986). In the present investigation we have determined the effect of NPY on the different components of the mechanical response of the vas deferens to single electrical pulses. The prostatic third of the vas deferens, obtained from freshly-killed albino Wistar rats, 200-250 g, was suspended between two parallel platinum electrodes in a 1 ml organ bath containing Krebs-Henseleit solution, bubbled with 95% O_2 + 5% CO_2 mixture, at 37°C. After allowing equilibration period of 30-45 min the preparation was subjected to electrical field stimulation (frequency: 16.6 mHz, Current: 690 mA) and the changes in tension were recorded isometrically and displayed on a storage oscilloscope. With stepwise increase in pulse-duration from 0.01 to 0.08 ms, the tension developed in response to electrical stimulation, displayed a corresponding increase peaking at 0.03 ms and remained constant between 0.03 and 0.08 ms pulse-duration (component A). With further stepwise increase from 0.09 ms to 1.0 ms the tension developed by the preparation grew paralleling the rise in pulse-duration up to 0.4 ms and remained steady thereafter up to 1.0 ms. This further rise in tension superimposed on component A has been labelled as component B. Additional increases in the pulse duration evoked a decline in the twitch tension; the decline peaked at 1.8 ms (component C).

NPY 0.25 μM produced a modest inhibition of component A and a moderate potentiation of component B (mean% ± s.e. mean of the control values of A and B respectively; 60 ± 9 and 140 ± 19, n = 7). Treatment with α-blocker phentolamine, 10μM did not alter qualitatively the effect of NPY on components A and B. There is good evidence for the presence of a nifedipine-resistant adrenergic element in the motor transmission to the rat vas deferens (McGrath, 1978; French & Scott, 1981). In order to study the effect of NPY on the adrenergic motor transmission and the inhibitory transmission (component C), components A and B were blocked by nifedipine, 10μM. NPY, 0.25μM depressed both the adrenergic motor component and the inhibitory component C (mean % of the control response ± s.e. mean: adrenergic motor component : 59 ± 10; component C: 54 ± 7; n = 4).

The above results indicate that exogenously applied NPY inhibits the adrenergic component together with components A and C in the response of the prostatic end of rat vas deferens to single pulse stimulation. Component B is potentiated by NPY giving credence to the possibility that this component might be due to the release of endogenous NPY in this tissue.

French,A.M. & Scott,N.C. (1981) Br.J.Pharmac. 73, 321-323
Huidoro-Toro,J. et al (1985) Eur.J.Pharmac. 109, 317-318
Lundberg,J.M. et al. (1982) Acta physiol.Scand. 116, 477-480
Lundberg,J.M. & Stjarne,L. (1984) Acta Physiol.Scand. 120, 477-479
McGrath,J.C. (1978) J.Physiol. 283, 23-39
Zar,M.A. (1986) Br.J.Pharmac. 87, 213P

STIMULANT AND ANTAGONISTIC ACTIONS OF OPIATE DRUGS ON THE RAT VAS DEFERENS.

R.M.Botting and P.Kent, Department of Biological Sciences, North East Surrey College of Technology, Ewell, Surrey, UK.

Electrically induced twitches of the isolated rat vas deferens can be reduced in size by β-endorphin, leucine enkephalin, methionine enkephalin and etorphine. Many opiate drugs on the other hand, including morphine, do not share this action, but cause an increase in twitch height (Huidobro et al, 1980). It is also possible to show antagonism of β-endorphin by morphine and some benzomorphans (Gillan et al, 1981). The opiate receptors in the rat vas deferens have been designated, ε receptors, selective for β-endorphin (Schulz et al, 1979).

Isolated vasa deferentia from Sprague Dawley rats (NESCOT strain) were suspended in Krebs bicarbonate saline and stimulated with single pulses of 1 ms at 0.1 Hz and supramaximal voltage. The effects of ethylketazocine, meptazinol, pethidine, morphine, bremazocine, and naloxone were investigated on the normal twitch and on the twitch reduced by between 60 and 95% with the opiate, etorphine or the α_2 receptor agonist, clonidine. Concentrations of etorphine, 0.24 - 2.43 μM and clonidine, 13.04 - 43.47 nM were used (see Table 1).

Table 1. Concentrations of opiate to increase the twitch or reverse inhibition by 50%. Mean ± s.e. mean x 10^{-6}M.

Drug	Alone	vs etorphine	vs clonidine
Ethylketazocine	17.56± 5.55 (7)	13.98± 7.35 (5)	36.83± 17.43 (4)
Meptazinol	41.91±6.70 (4)	47.23±11.14 (3)	36.29± 7.52 (4)
Pethidine	99.05±53.10 (4)	110.75±26.13 (3)	58.88±8.85 (3)
Morphine	>500 (4)	69.93±41.5 (4)	>300 (5)
Bremazocine	>500 (3)	1.80±0.40 (6)	62.39±7.57 (4)
Naloxone	>500 (4)	2.82±1.13 (3)	>350 (4)

For ethylketazocine, meptazinol and pethidine, similar concentrations were needed to potentiate the twitch by 50% and reverse both etorphine and clonidine inhibition by 50%. On the other hand, morphine and bremazocine were more potent in reversing etorphine-induced inhibition than potentiating the twitch or reversing clonidine induced inhibition. Naloxone reversed etorphine inhibition only, but did not potentiate the twitch or reverse clonidine-induced inhibition.

It seems likely therefore, that bremazocine is a potent and morphine a weak antagonist on the opiate receptor in the rat vas deferens and that other opiates appear to antagonise because of their action in increasing twitch height. Bremazocine may also possess some antagonist action at α_2 receptors.

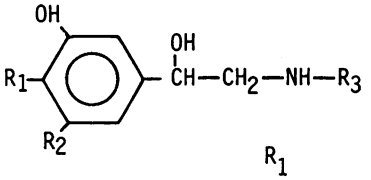
Gillan, M.C.G., Kosterlitz, H.W. and Magnan, J. (1981) Br.J.Pharmac. 72, 13-15.
Huidobro, F., Huidobro-Toro, J.P. and Miranda, H. (1980) Br.J.Pharmac. 70, 519-525.
Schulz, R., Faase, E., Wuster, M. and Herz, A., (1979) Life Sci. 24, 843-850.

Acknowledgement is made of gifts of meprazinol (Dr A White, Wyeth Labs), bremazocine (Dr D Romer, Sandoz), ethylketocyclazocine (Dr A E Soria, Sterling-Winthrop) and etorphine (Reckitt and Colman).

THE PREJUNCTIONAL β-ADRENOCEPTOR OF THE EPIDIDYMAL PART IN RAT VAS DEFERENS.

A. Bast, H. Timmerman & O.P. Zuiderveld, Department of Pharmacochemistry, Subfaculty of Chemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands.

Putative (patho)physiological functions of prejunctional β-adrenoceptors are manifold. Design of selective agents for prejunctional β-adrenoceptors may therefore constitute an important goal. In this study the prejunctional β-adrenoceptor activity of different β-adrenoceptor agonists (Fig. 1) were compared. We employed a field stimulated rat vas deferens, pulsed at low frequencey (0.1 Hz). The potency of the evoked twitches are considered as a measure for the amount of endogeneous released noradrenaline. Previous studies have shown that low concentrations of (-)isoproterenol inhibited the twitch response whereas higher concentration enlarged the stimulation evoked twitches.



	R_1	R_2	R_3
isoproterenol	OH	H	iC_3H_7
th 1206	OH	H	tC_4H_9
orciprenaline	H	OH	iC_3H_7
terbutaline	H	OH	tC_4H_9
CC 43	OH	H	p-OH-C_6H_4-C_2H_4
CC 25	OH	H	p-OH-C_6H_4-iC_3H_6
Me 454	OH	H	p-OH-C_6H_4-tC_4H_8
Me 641	H	OH	p-OH-C_6H_4-C_2H_4
fenoterol	H	OH	p-OH-C_6H_4-iC_3H_6
Me 506	H	OH	p-OH-C_6H_4-tC_4H_8

Fig.1 Compounds used in this study.

Vasa deferentia were obtained from Wistar rats (± 300 g). The epididymal portion was separated and placed between platinum electrodes under 0.4 g tension in Krebs solution (pH 7.4, 37°C, 95% O_2 / 5% CO_2). Contractions were obtained by low frequency field stimulation (0.1 Hz, 1 msec, 50-60 V) and recorded isotonically. Only one dose response curve was constructed at each preparation.
Compounds with ethyl and isopropyl N-substituents potentiated the evoked twitches with p-hydroxyphenylisopropyl > isopropyl > p-hydroxyphenylethyl. N-substitution with t-butyl and p-hydroxyphenyl-t-butyl gave compounds that only caused depression of the field stimulation induced contractions. Compounds with a catechol moiety were more active than compounds with a resorcinol group.
We observed minimal prejunctional β-adrenergic effects of t-butyl analogues despite their reported potent postjunctional β-adrenergic activities.

Our data suggest that the characteristics of the rat vas deferens prejunctional β-adrenoceptors do not correspond exactly with either of the postjunctional β-adrenoceptor subgroups.

DIFFERENT α-ADRENOCEPTORS MEDIATE TONIC AND RHYTHMIC CONTRACTIONS OF THE RAT VAS DEFERENS.

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

Spontaneous rhythmic contractions occur in the rabbit vas deferens following sexual activity; vasal contents being transported to the epididymis (Prins and Zaneveld, 1980). This suggests spontaneous rhythmic activity is involved in regulation of sperm reserves. In the rat vas deferens, Hay & Wadsworth (1983) showed α-adrenoceptor agonists produce an initial tonic response followed by rhythmic contractions. This initial tonic response to exogenous α-agonists (including noradrenaline, NA) is thought to be mediated via a single population of α_1-adrenoceptors (Leedham & Pennefather, 1982). However, it is not clear whether the population of α-adrenoceptors involved in the mediation of rhythmic contractions is the same as that involved in the tonic contraction. In this study, we show that tonic and rhythmic contractions are mediated by separate populations of α-adrenoceptors.

Vasa deferentia from Spraque-Dawley rats (200-300g) were set up in Mg^{2+}-free Tyrodes bubbled with 95% O_2:5% CO_2. Some rats received chronic treatment with guanethidine (injected i.p. 25 mg/kg per day 5 days a week for 6 weeks) in order to destroy adrenergic nerves (Lafi & Leake, 1986). Control concentration of agonists produced a 50% maximal tonic response.

Exogenous NA (3μM), dopamine (DA, 17.5μM) and the α_1-agonist methoxamine (MX, 5μM) produced tonic responses followed by rhythmic activity in untreated vasa. At comparable doses DA-induced rhythmic responses were bigger than those induced by NA, whilst rhythmic activity produced by MX lasted for hours. Chronic denervation markedly potentiated the tonic contraction, but reduced rhythmic responses to NA; had little effect on the tonic response to DA but markedly potentiated rhythmic activity: neither MX-induced response was affected. In denervated vasa, the tonic response to MX (5μM) was completely abolished by phentolamine (0.01μM) and verapamil (1μM) whereas the response to DA (15μM) and NA (0.15μM) were only inhibited to about 20%. This residual component was only abolished at higher doses (≃ x25) of antagonists. Both DA-and MX- induced rhythmic responses were resistant to the low dose of antagonists and were only abolished at high doses i.e. phentolamine (1μM) and verapamil (100μM). The α_1-antagonist prazosin (0.001μM) abolished DA-induced tonic responses but, at doses up to 0.1μM, failed to completely abolish DA-induced rhythmic responses. Lowering bath temperature to 20°C inhibited rhythmic activity induced by DA and MX but potentiated tonic responses induced by the agonists.

These results suggest that DA-and MX- induced tonic and rhythmic responses are mediated via postsynaptic α-adrenoceptors, as both types of activity can be inhibited by α-antagonists. 80% of the tonic response to DA and NA is more sensitive to the antagonists than the remaining 20%, indicating that DA and NA produce tonic responses through activation of more than one population of α-receptors showing differential sensitivities to antagonists. Furthermore DA-and MX- induced tonic contractions show much higher sensitivity to the antagonists than does rhythmic activity induced by the same agonists. This indicates that tonic and rhythmic activities are also mediated by at least two populations of α-adrenoceptor, which can be distinguished by lowering bath temperature.

Hay, D.W.P. & Wadsworth, R.M. (1983) Br. J. Pharmac., 79, 347-362
Lafi, M.A.K. & Leake, L.D. (1986) Br. J. Pharmac., 87, 147p
Leedham, J.A. & Pennefather, J.N. (1982) Br. J. Pharmac., 77, 293-299
Prins, G.S. & Zaneveld, L.J.D. (1980) Biol. Reprod., 23, 904-999

THE EFFECT OF TEMPERATURE ON α_1-AND α_2-ADRENOCEPTOR SENSITIVITY IN RAT VAS DEFERENS.

A.M. French, G.C. Jefferson and F.M. McKean, Department of Pharmacy, Heriot-Watt University, Edinburgh EH1 2HJ.

It has been reported that a reduction in temperature causes a functional subsensitivity of post-junctional alpha two but not alpha one adrenoceptors in dog saphenous vein (MacAdams & Waterfall 1984). The purpose of this study was to examine the effect of temperature on pre-junctional alpha two and post-junctional alpha one adrenoceptors in rat vas deferens.

Rat vasa deferentia were removed and bisected in the ratio 60:40, epididymal: prostatic. Pairs of prostatic and epididymal ends were separately mounted, under 0.5g tension, in Krebs-Henseleit at 37°C and gassed with 95% O_2/5% CO_2. Tissues were allowed to equilibrate for 60 minutes before drug additions were made. The prostatic portions were field stimulated by single, supramaximal pulses (1.0ms, 300mA) at 5 minute intervals. Contractions were recorded isometrically using UF1 transducers coupled to a computerised data acquisition system. Cumulative dose-reponse curves were prepared for xylazine (XYL) and BHT 933. Similar curves were constructed for XYL in the presence of prazosin. This procedure was repeated at 27, 20 and 15°C. ID_{50} values for the agonists were calculated at each temperature. For the epididymal portions of tissue, contractions were recorded isotonically. Dose-response curves were constructed for noradrenaline (NA) and phenylephrine (PE) at 15, 20, 27 and 37°C. Responses were expressed as a percentage of the maximum response at each temperature and ED_{50} values were calculated.

Agonist / Temp.	37	27	20	15
		ID_{50} (+- s.e.)		
Xylazine (nm)	11.3 +- 8.50	44.3 +- 3.0	500.0 +- 8.50	6230.0 +- 8.50
Xylazine + Prazosin (nm)	11.3 +- 8.50	30.3 +- 9.3	200.0 +- 9.10	350.0 +- 8.90
BHT 933 (nm)	102.0 +- 36.0	----------	----------	400.0 +- 50.0
		ED_{50} (+- s.e.)		
Noradrenaline (um)	5.0 +- 0.87	3.9 +- 0.92	4.1 +- 0.82	3.5 +- 0.76
Phenylephrine (um)	16.7 +- 0.86	----------	----------	2.6 +- 0.21

$P < 0.05$ for all values excepting NA 27:20°C and 20:15°C

Within the temperature range 37 - 15°C, there was a slight increase in tissue sensitivity to NA and PE and a decrease in tissue sensitivity to XYL and BHT 933. The reduction in tissue sensitivity to XYL was less pronounced in the presence of prazosin. The slight increase in tissue sensitivity to NA and PE may be explained by inhibition of the uptake mechanism at low temperature, as previously demonstrated in the mouse vas deferens by Buckner, Bohuski & Ryan (1975). XYL is not a selective alpha two agonist and also has some alpha one effects. A potentiating effect of XYL on the prostatic portion of rat vas deferens, mediated by alpha one adrenoceptors, has previously been reported (French & Henderson 1985). Potentiation was found to increase as the temperature was decreased therefore the ID_{50} values calculated are probably not the true values since the alpha two inhibitory effect is offset by the facilitatory alpha one effect. It is likely that more realistic ID_{50} values are obtained when prazosin is present to block the facilitatory alpha one component of the response.

Thus it may be concluded that temperature reduction produces a significant decrease in responsiveness of alpha two but not alpha one adrenoceptors in rat vas deferens.

Buckner C.K.,Bohuski K. & Ryan C.F.(1975) Arch.Int.Pharmacodyn.Ther.216, 19-27.
French A.M. & Henderson D.J.(1985) Br.J. Pharmac. 86, 510P.
MacAdams R.P. & Waterfall J.F. (1984) Br.J.Pharmac. 83, 412P

LOSS OF β-ADRENOCEPTORS, PRESENT ON THE SMOOTH MUSCLE OF THE RAT VAS DEFERENS, WITHOUT DESENSITIZATION.

E.T. Bakker[2], A. Bast[1], B. Rademaker[3], K.J.H. van Buuren[2], K. Wezel[1], [1]Dept. of Pharmacochemistry, Vrije Universiteit, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands,
[2]Dept. of Pharmacology and Pharmacotherapy, State University of Utrecht, Catharijnesingel 60, 3511 GH Utrecht, The Netherlands.
[3]Duphar B.V., Dept. of Pharmacology, section G.I., C.J. van Houtenlaan 36, 1381 CP Weesp, The Netherlands.

We have characterised β-adrenoceptors in rat vas deferens both directly by monitoring specific Jodocyanopindolol ([^{125}I]ICYP) binding and indirectly by measuring the inhibition of electrically-evoked contractions. The rat vas deferens was used as a model system, in order to be able to compare the binding properties of β-adrenoceptors in membrane preparations to the properties of the β-adrenoceptors mediating a functional response in the intact tissue.

Specific binding for [^{125}I]ICYP showed a high affinity dissociation constant (15 pM) and a maximal capacity of 160 pmol/mg protein. β_1-, β_2-subtype selective antagonists, ICI 89.406 (β_1) and ICI 118.55 (β_2), inhibited [^{125}I]ICYP binding revealing 13% β_1 and 87% β_2 adrenoceptors. No significant differences were detected between the epididymal part and the prostatic part of the vas deferens.

(-)Isoproterenol also displaced [^{125}I]ICYP binding from two sites (n_H : 0.61); the first one (K_H) with a K_d of $3.8.10^{-8}$ M and the second one (K_L) with a K_d of 11.10^{-6} M.

After in vitro administration of (-)isoproterenol to the intact vas deferens, we subsequently observed down-regulation of approximately 46% of the β-adrenoceptors within 45 min and a reappearance to control value of these binding sites during the next 3 hrs in the presence of (-)isoproterenol.

Down-regulation is a receptor-mediated event and cannot be explained as a result of "tight-binding" of (-)isoproterenol to β-adrenoceptors (Nerme et al, 1985), since there was not change in the number of β-adrenoceptors when smooth muscle membranes of the vas deferens were preincubated with (-)isoproterenol (10^{-4} M), both in the absence or presence of GTP (10^{-4} M). However, we found no significant shift in the ratio β-adrenoceptors being in the high affinity state (K_H) and the low affinity state (K_L) during down-regulation. Neither did we find a decrease of maximal (-)isoproterenol relaxation potency in functional experiments, after pre-incubation with isoproterenol.

Hence it can be concluded that no desensitization has taken place which is in accordance with the conclusions of May et al (1985), in spite of the loss of receptors; this discrepancy can be explained by the existence of spare receptors.

Nerme, V., Severne, Y., Abrahamsson, T. & Vauquelin, G. (1985) Bioch. Pharmac. 34, 2917-2922.
May, J.M Abel,P.W. & Minneman, K.P. (1985) Naunyn-Schmiedeberg's Arch. Pharmac, 331, 324-333.

THE INDUCTION OF PULMONARY AUTONOMIC RECEPTOR DEFICIENCIES BY ANAPHYLACTIC MEDIATORS AND PROTEOLYTIC ENZYMES.

C. Beneker, E. de Boer, H.J. Harmsen, J.A.M. Raaijmakers and A.J. van Rozen.
Department of Pulmonary Diseases, State University Hospital Utrecht, Utrecht, The Netherlands.

In previous studies we have shown autonomic receptor defects in pulmonary tissue of COLD patients (Raaijmakers et al, 1984) . This supports the view that such deficiencies may have a part in the pathogenesis of the disease. However, the defects shown in these studies are not uniform in all COLD patients (Raaijmakers et al, 1985).

The aims of this study are possible mechanisms by which the observed defects may be induced. The experiments were performed on chopped lung tissue, and deal with the effects on pulmonary autonomic receptors of histamine, PAF, arachidonic acid metabolites such as leukotrienes and HETE's. Enzymes such as elastase were used to study the possible influence of proteolytic enzymes on receptor characteristics in patients with emphysema.

In the experiments concerning the role of anaphylactic mediators in this system we studied the influence of the addition of individual mediators on receptor parameters but also on the synthesis and release of other mediators. Receptor characteristics were determined by radioligand binding assays using ^{3}H-prazosin, ^{3}H-DHA and ^{3}H-QNB to label α- and β-adrenoceptors, and muscarinic cholinergic receptors respectively.

The results indicate an influence of anaphylactic mediators and enzymes on pulmonary autonomic receptors as well as an influence of individual mediators on the synthesis and release of other mediators. Furthermore it appears that the proteolytic enzymes that play a part in the pathogenesis of emphysema, may also be responsible for the receptor defects that were demonstrated in the pulmonary tissue of emphysema patients.

Raaijmakers et al., Clinical Science 66 (1984) 585-590.
Raaijmakers et al., Progress in Repiration Research. Vol 19 (1985), pp 153-158.

This project is supported by NAF grant no. 84.13.

MICE TRACHEAL β-ADRENERGIC AND CHOLINERGIC RECEPTOR FUNCTION AND ITS DISTURBANCE BY BORDETELLA PERTUSSIS AND ITS COMPONENTS.

G.H. Veenendaal, D.J. Kool, F.P. Nijkamp and D.J. de Wildt[1], Institute for Veterinary Pharmacology, Pharmacy and Toxicology, University of Utrecht, Dept. of Pharmacology, P.O. Box 80176, 3508 TD Utrecht, The Netherlands; [1]National Institute of Public Health and Environmental Hygiene, Bilthoven, The Netherlands.

Vaccination of laboratory animals with Gram negative bacteriae like Bordetella pertussis can produce a variety of biological effects (Schreurs et al., 1983; De Wildt et al., 1983). Previous experiments with guinea pigs show that pretreatment with B. pertussis diminished β-adrenoceptor function and number in the respiratory system (Van Heuven-Nolsen et al., 1986). The aim of our present study was to find out if vaccinating mice with B. pertussis resulted in a similar modulation of contractile cholinergic and/or relaxing β-adrenergic mechanisms. Moreover we investigated which bacterial cellwall component, intact endotoxin (LPS) or lymphocytosis promoting factor (LPF), was responsible for the observed phenomena in mice isolated tracheal smooth muscle preparation. Finally a comparison was made between the effects of B. pertussis vaccination in normal and in nude mice (LP CPB nu/nu). Mice were pretreated 3 days prior to the in vitro experiment with either B. pertussis organisms or with saline (i.p.).
In the normal mouse $2x10^9$ organisms/100 g b.w. B. pertussis i.p. caused as compared to the control group tracheal hyperreaction to the cholinergic agonist carbachol (190%); 10^{10} organisms/100 g caused attenuation of contraction (65%). In the nude mouse also hyperreaction to carbachol occurred (231%). However a higher dose was required ($5x10^{10}$ organisms/100 g). In the normal mouse an attenuation of tracheal smooth muscle relaxation by isoprenaline (22%) could be demonstrated after pretreatment with 10^{10} organisms/100 g. In the nude mouse different doses were ineffective.
LPS injection induced in the normal mouse hyperreaction after carbachol (136%) comparable to the effect of $2x10^9$ organisms/100 g B. pertussis in the normal and $5x10^{10}$ organisms/100 g in the nude mouse. A four times higher dose of LPS caused attenuation by 42% of the maximal carbachol induced contraction in the normal mouse. The relaxation induced by isoprenaline tended to be reduced.
Pretreatment i.p. of normal mice with LPF caused hyperreaction (153%) to carbachol but was ineffective on isoprenaline induced relaxation.
Our results indicate that the cellwall component LPS and probably not LPF is responsible for inducing β-adrenoceptor impairment by B. pertussis organisms in mice. This is in accordance with results obtained previously with guinea pigs (Van Heuven-Nolsen et al., 1986). The thymus does not seem to influence the carbachol hyperreactivity to B. pertussis. A role for the thymus in modulating β-adrenoceptor activity cannot be excluded.

De Wildt, D.J., Kreeftenberg, H.G. and Nijkamp, F.P. (1983) Infect. Imm. 41, 476.
Schreurs, A.J.M., Verhoef, J. and Nijkamp, F.P. (1983) Eur. J. Pharmacol. 87, 127.
Van Heuven-Nolsen, D., Folkerts, G., De Wildt, D.J. and Nijkamp, F.P. (1986) Life Sciences 38, 677.

KETANSERIN IS A STRONG INHIBITOR OF THE ANAPHYLACTIC BRONCHOCONSTRICTION IN THE RAT.

M. Ottenhof & J.G.R Ufkes, Department of Pharmacology, Academic Medical Centre, University of Amsterdam, Meibergdreef 15, 1105 AZ Amsterdam, The Netherlands.

Using a recently developed method for inducing IgE-mediated, bronchial and cardiovascular anaphylaxis in Brown-Norway rats, five different types of anti-allergic agents were tested with regard to mortality, bronchoconstriction and cardiovascular events (Ufkes & Ottenhof, 1984). With the exception of the histamine H_1-receptor antagonist mepyramine (no activity at all), each anti-allergic agent tested showed a different and characteristic profile of anti-allergic activity. Several studies indicate that 5-hydroxytryptamine (5-HT), possibly together with cyclo-oxygenase and lipoxygenase products, is a mediator of the bronchial anaphylaxis in actively sensitized rats. In a recent study, using rat isolated lungs, it was established that considerable amounts of histamine, 5-HT and SRS-A were released during the antigen-induced bronchoconstriction (Ottenhof et al., 1985). Within this scope the present experiments were designed to determine the effects of ketanserin, a potent and selective antagonist of 5-HT at 5-HT_2 receptors (Leysen et al., 1981) on the IgE-mediated anaphylaxis in vivo.

Sensitization procedure, measurement of bronchial and cardiovascular function, antigen challenge and analysis of data were performed as described previously (Ufkes et al., 1983). Ketanserin was administered in doses of 2 and 20 µg/kg i.v. 10 min before the antigen challenge.

The influence of both doses ketanserin on the anaphylaxis is summarized in Table 1.

Table 1 The effect of ketanserin on mortalityrate, bronchoconstriction and cardiovascular events as compared to controls

	Dose µg/kg	n	MORTALITY RATE Rats (n)	Time to death (min)	BRONCHOCONSTRICTION C_{dyn}- decrease (%)	R_1- increase (%)	CARDIOVASCULAR BP fall (%)	Recovery time min (n)
Controls		12	11	113 ± 13	100 ± 11	100 ± 10	74 ± 2	99 (1)
Ketanserin	2	6	5*	53 ± 9□	69 ± 12△	84 ± 22*	77 ± 2*	50 (1)
	20	6	6*	61 ± 9□	21 ± 5◇	29 ± 11◇	79 ± 2△	-

* not significant, △ $p<0.05$, □ $p<0.01$, ◇ $p<0.001$.

It can be seen that ketanserin did not influence the mortality rate but affected the time to death negatively. The antigen-induced bronchoconstriction was dosedependently suppressed to a large extent, whereas the initial fall in blood pressure was slightly increased.

This study was supported by a research grant from the Netherlands Asthma Foundation (NAF 82.15).

Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vanderberk, J. & Janssen, P.A.J. (1981) Life Sci., 28, 1015-1022.
Ottenhof, M., Ufkes, J.G.R. & Van Rooy, H.H. (1985) Br.J. Pharmac., 86, 627-636.
Ufkes, J.G.R. & Ottenhof, M. (1984) J.Pharmac.Meth., 11, 219-226.
Ufkes, J.G.R., Ottenhof, M. & Aalberse, R.C. (1983) J.Pharmac.Meth., 9, 175-181.

EVIDENCE FOR "5-HT_1-LIKE" RECEPTORS IN HUMAN UMBILICAL ARTERY (HUA).

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

At physiological pO_2 (15mmHg) contractions induced by 5-HT in HUA are via 5-HT_2 receptors. However, at high pO_2 (120mmHg) there is a ketanserin resistant "1st phase" component (McGrath et al, 1985, 1986). Here we set out to determine if the ketanserin-resistant component could be ascribed to a "5-HT_1-like" receptor.

Isometric tension was recorded from longitudinal strips of artery in Krebs at 37°C. Cumulative concentration-contractile response curves (CRC) to 5-HT, 5-CT and methysergide were constructed at two pO_2's : (i) 15mmHg (low pO_2) ; (ii) 120mmHg (high pO_2). Potency of 5-CT, relative to 5-HT was determined at low and high pO_2 in separate sets of experiments: cumulative CRC's to 5-HT followed by 5-CT were constructed. Potencies were calculated at the level of EC_{25} and EC_{50}. For the experiments with methysergide one arterial strip acted as a control and antagonists were added to three other strips since tachyphylaxis occurs.

The potency of 5-CT, relative to 5-HT, is shown in Table 1.

Table 1	5-HT CRC		EC_x5-CT/EC_x5-HT		5-CT CRC			n
	EC_{25} (nM)	EC_{50} (nM)	EC_{25}	EC_{50}	EC_{25} (nM)	EC_{50} (nM)	% of 5-HT maximum	
pO_2 (mmHg)								
13±1	18	40	0.64	7.1	13	130	74±13	5
123±1	3.3	9.8	0.15	2.7	0.25	34	88±8	6

Values are geometric means.

At low pO_2 there was a 1st phase of the 5-CT CRC at low concentrations, which was increased at high pO_2 but was blocked by indomethacin (1uM). At high pO_2 these 1st phase responses were, in different preparations, either maintained or transient.

At low pO_2 methysergide showed no agonism (10nM-1uM) (n=6). At high pO_2 methysergide contracted HUA between 10nM and 1uM (EC_{50}=0.15uM) (geometric mean, n=7). The EC_{50} for 5-HT at high pO_2 was 9.8nM (non-paired tissues). Thus 5-HT was approximately 15 fold more potent than methysergide. The maximum response to methysergide was 64±18% of a 50mM KCl contraction. In contrast the 5-HT maximum (relative to a 50mM KCl contraction) was 222±26% (non-paired tissues). Neither prazosin (30nM) nor ketanserin (30nM) shifted the methysergide CRC or reduced the maximum response. Indomethacin (1uM) did not significantly shift the methysergide CRC but significantly reduced the maximum response to 43±11% (relative to a 50mM KCl contraction).

The agonist profile found here provides evidence that the 5-HT receptor in HUA (in addition to the 5-HT_2 receptor), whose expression depends on a high pO_2, may be "5-HT_1-like". At high pO_2 5-CT was more potent than 5-HT (at low concentrations only) and methysergide was found to be an agonist. These findings are similar to those in dog saphenous vein (Feniuk et al, 1985) and rabbit basilar artery (Bradley et al, 1986) which contain a 5-HT receptor described as "5- HT_1-like". In HUA the "5-HT_1-like" receptor has a prostaglandin link.

The authors wish to thank The Scottish Hospital Endowments Research Trust for financial support and Glaxo for the gift of 5-CT.

Bradley, P.B. et al (1986) Br. J. Pharmac. 87, 3-4.
Feniuk, W. et al (1985) Br. J. Pharmac. 86, 697-704.
McGrath, J.C. et al (1985) Br. J. Pharmac. 84, 199-202.
McGrath & MacLennan (1986) Br. J. Pharmac. 88, 320P.

CHARACTERISATION OF HUMAN PLACENTAL ADRENOCEPTORS.

J.G. Milligan, J.G. Riddell and R.G. Shanks, Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Belfast, Northern Ireland

The human placenta is a rich source of adrenoceptors (Shocken et al., 1980), but their classification is incomplete. We have used specific antagonists to classify the adrenoceptors of the human placenta, and to determine the relative number of both alpha and beta sub-types.

Cotyledons of fresh human placenta from normotensive mothers were dissected from the area close to the attachment of the umbilical cord. The cotyledons were homogenized at 4^0C in Hank's balanced salt solution (HBSS) as buffer, centrifuged twice at 3,000 g for 10 min at 4^0C and the pellet discarded. The resulting supernatant was centrifuged at 35,000 g for 30 min at 4^0C, and the pellet resuspended in ice-cold buffer at a protein concentration of approximately 1 mg/ml for use in membrane binding assays.

The ligands used in the binding assays were [^{3}H]dihydroalprenolol [^{3}H](DHA) (Amersham, 85 Ci/mmol), [^{3}H]prazosin (Amersham, 26 Ci/mmol, and [^{3}H]rauwolscine (Amersham, 78.5 Ci/mmol). All assays were carried out in duplicate for 30 min at 25^{o}C in a final volume of 1 ml (800 µl membrane suspension; 100 µl [^{3}H]ligand; and 100 µl buffer (total counts) or antagonist (non-specific counts)). Non-specific binding was determined using atenolol or ICI 118,551 (1 µM) for $beta_1$ and $beta_2$ adrenoceptors respectively, and by phentolamine (10 µM) for alpha-adrenoceptor assays. The incubation was ended by rapid vacuum filtration over Whatman GF/D glass fibre filters, which were washed with a total of 15 ml ice-cold saline. Radioactivity was measured by liquid scintillation counting using a Toluene/Triton-X cocktail. The results were analysed using a non-linear curve-fitting programme EBDA/LIGAND (Munson & Rodbard, 1980; McPherson, 1983).

[^{3}H]DHA bound to beta-adrenoceptors with high affinity, K_d=1.28 $\pm$ 0.2 nmol ($\pm$ s.e.m.); B_{max}=75.7 $\pm$ 11.4 fmol/mg protein ($beta_1$ receptors), K_d=1.67 $\pm$ 0.3 nmol; B_{max}=123 $\pm$ 18.7 fmol/mg protein ($beta_2$ receptors) (n=20). Binding was saturable, rapid, reversible, and exhibited specificity and stereo-selectivity. [^{3}H]prazosin did not bind specifically to human placental membranes. [^{3}H]rauwolscine bound to a single saturable receptor with K_d=5.5 $\pm$ 1.5 nmol, B_{max}=45.7 $\pm$ 8.2 fmol/mg protein (n=20). These binding sites displayed rapid, reversible binding, saturability, and appropriate specificity.

We conclude that the human placenta contains $beta_1$, $beta_2$ and $alpha_2$ adrenoceptors in the approximate proportion 5:8:3. No $alpha_1$ adrenoceptors were detected.

Shocken, D.D. et al. (1980). J. Clin. Endocrinol. Metab., 50, 1082-1088.
Munson, P.J. and Rodbard, D. (1980). Anal. Biochem., 107, 220-239.
McPherson, G.A. (1983). Computer Prog. in Biomed., 17, 107-114.

TRYPSIN-LIKE ACTIVITY IN SALIVARY GLANDS OF THE RAT.

A. ADAM*, J. DAMAS** and R. VRANCKX***.*Laboratoire de Radioimmunologie et **Laboratoire de Physiologie humaine, Université de Liège, B-4020 Liège, Belgium. ***Laboratoire de Biochimie, Faculté de Médecine Xavier Bichat, F-75018 Paris, France.

Rat plasma contains a third form of kininogen, T-kininogen or α_1-cystein proteinase inhibitor. This kininogen is a kinin-forming substrate for trypsin but not for kallikreins (Greenbaum, 1984; Furuto-Kato et al., 1985). The plasma of Brown Norway rats from the strain BN/May Pfd f contains this kininogen only (Hayashi et al., 1985; Damas and Adam, 1985). We observed that in these animals rat salivary gland homogenate induced a tachyphylactic hypotensive response, mimicking kinin release (Damas and Adam, 1980), and that this homogenate acting on the BN rat plasma released a kinin-like activity (Damas and Adam, 1985). These glands could, thus, exert a trypsin-like activity. We started the characterization of this activity.
The submandibular glands from normal Wistar rats were excised. A 20 % homogenate was prepared in NaCl (0.15 M) using a Potter homogenizer, incubated at room temperature with Triton X-100 (1 %) for 30 min, centrifuged at 5000 g for 15 min and stored at -20°C until assay. The citrated plasma from BN rats was brought to pH 2 by HCl and incubated for 15 min at room temperature. The acidified plasma was then neutralized with NaOH and 0.15 M Tris HCl (pH 8). For the determination of the kinin-forming activity of the salivary glands, the plasma (0.2 ml) was incubated at 37°C for 15 min with Tris HCl (0.15 M, pH 8), EDTA (2 mg/ml) and 20 mg/ml of the homogenate . Kinin release was determined by bioassay.
α_1-cystein proteinase inhibitor was purified according to Esnard and Gauthier (1983). Trypsin released from this protein a kinin-like activity: 11.5 $\pm$ 0.7 µg bradykinin equivalent per mg of protein. The homogenate of salivary glands released similar amounts of kinins: 11 $\pm$ 0.4 µg bradykinin equivalent per mg of protein.
The kinin-forming activity of the homogenate of salivary glands on BN plasma was inhibited by benzamidine (0.5 mM) SBTI (25 µM) aprotinin (600 U/ml) and DFP (10 mM), but was not modified by trypsin inhibitor from chicken egg white (10 µM).
The homogenate (4 ml) was loaded on Sephadex G-100 column (58x1.25 cm) equilibrated with NaCl (0.15 M). Tissue kallikrein (amidase activity) in the eluate was measured in the presence of SBTI (25 µM) using the chromogenic substrate H-D-val-leu-erg-pNA. The kinin-forming activity of salivary glands on BN plasma was eluted from the column in a single peak which did not correspond to a protein peak. It was eluted in the same volume as tissue kallikrein. The release of kinin from purified T-kininogen and the inhibition of this kinin-forming activity by SBTI suggests that salivary glands contain a trypsin-like activity, beside tissue kallikrein.

Damas, J. & Adam, A. (1980) Experientia 36, 586-587.
Damas, J. & Adam, A. (1985) Mol. Physiol. 8, 307-316.
Esnard, F. & Gauthier, F. (1983) J. Biol. Chem. 258, 12443-12447.
Greenbaum, L.M. (1984) Biochem. Pharmacol. 33, 2943-2944.
Hayashi et al. (1985) Thombosis Res. 39, 313-321.
Furuto-Kato et al. (1985) J. Biol. Chem. 260, 12054-12059.

INTERACTION BETWEEN THE EFFECTS OF SUBSTANCE P AND ISOPRENALINE ON Ca^{++} METABOLISM IN THE ISOLATED RAT PAROTID SALIVARY GLAND.

S. Arkle, I. Fairbrother[1], R. Michalek[1] & D. Templeton[1], School of Pharmacy, Portsmouth Polytechnic, Portsmouth and [1]Department of Physiology and Pharmacology, University of Southampton, Southampton SO9 3TU.

Secretion of amylase from the rat parotid salivary gland can be evoked by Substance P, acetylcholine and α-adrenoceptor agonists by a mechanism which requires extracellular Ca^{++}. β-adrenoceptor stimulation, on the other hand, is dependent on the release of Ca^{++} from an intracellular site. Simultaneous stimulation of both mechanisms results in a potentiation of the secretory response (Arkle et al, 1985) and in the present paper we have investigated the role of Ca^{++} in this effect using both $^{45}Ca^{++}$ efflux and quin-2 fluorescent studies.

The ventro medial portions of both parotid glands from a male rat, killed by cervical dislocation, were chopped into small (0.5 mm) slices by hand and incubated at 37°C in Krebs Henselheit buffer containing $^{45}Ca^{++}$ (2.5 μCi/ml) for 60 min. After loading with ^{45}Ca, the tissue was placed in a 1 ml perfusion chamber maintained at 37°C and the perfusate (2.2 ml min^{-1}) collected at one min intervals for scintillation counting. A 90 min washout period was used to remove any extracellular $^{45}Ca^{++}$ before the tissue was exposed to drugs

Efflux rates are expressed as the fraction of the ^{45}Ca released during the test period relative to the immediately preceding control period. In control experiments the efflux rate remained constant indicating that the tissue slice preparation was stable for the duration of the experiment. Addition of either Substance P (SP) or isoprenaline (IPR) caused an increase in ^{45}Ca efflux which was dose dependent with a threshold at 10^{-6} M for SP and 10^{-7} M for IPR. However when SP was applied simultaneously with a subthreshold dose of IPR (2×10^{-9} M) the threshold was reduced to 10^{-8} M and the responses to larger doses of SP were significantly increased.

Table 1 Effect of SP and IPR on ^{45}Ca efflux (area under normalised ^{45}Ca efflux curve, arbitrary units)

Dose (M)	10^{-8}	10^{-7}	10^{-6}	10^{-5}
Control	9628	9630	9344	9671
SP	10126	9535	10303	10785
SP + IPR (2×10^{-9})	10279	10879	12530	12134
p	N.S.	0.012	0.003	0.029

In separate studies the level of cytosolic free ionic Ca^{++} was measured in isolated acini using quin-2 (Rink & Pozzan, 1985). The cytosolic free Ca^{++} activity in unstimulated acini was 192 ± 9 nM (n = 111). Both SP and IPR produced a dose-dependent increase in free Ca^{++} to maximum values of 446 ± 86 nM (n = 6) and 246 ± 49 nM (n = 14) respectively. The effects of combined exposure to SP and isoprenaline are currently under investigation.

Arkle, S., Iversen, L.L., Michalek, R., Poat, J.A. & Templeton, D. (1985) Br.J. Pharmac. 86, 701P.

Rink, T.J. & Pozzan, T. (1985) Cell Calcium 6, 133-144.

ABSENCE OF A DIRECT EFFECT OF ADENOSINE ON SKINNED AORTIC FIBRES.

C.J. Long and T.W. Stone, Department of Physiology, St. George's Hospital Medical School, University of London, London SW17 ORE, U.K.

It is generally acknowledged that activation of the contractile apparatus is dependent on increased levels of free intracellular calcium. Such calcium may originate from an extracellular source and/or an intracellular store(s) and the mechanisms by which this increased flux occurs are potential sites of action for interference by vasoactive agents. Adenosine has been known to possess vaso-relaxant activity for many years (Drury and Szent-Gyorgyi, 1929). The basis of the mechanism is still unclear. We have recently shown that adenosine appears to act via an action upon calcium and that this action may be preferentially directed against calcium of intracellular origin as compared to that of extra-cellular origin (Long and Stone, 1986). This work was therefore initiated to clarify the actions of adenosine and in particular to determine whether adenosine has a direct effect on the contractile proteins.

Male Wistar rats (250-300g) were killed by cervical dislocation and the thoracic aorta was removed and cut into a helical strip. Skinned fibres were prepared using Triton X-100 by the method of Ruegg et al. (1983). The fibres were mounted in a 1 ml organ bath and bathed in an initially Ca-free solution. Adjustments in Ca were made by wholly replacing this bathing solution with a Ca-containing one. Ca was buffered in all solutions with EGTA (4mM). Calmodulin (4μM) was also routinely present in all solutions.

In the absence of extracellular free Ca the skinned fibres exhibited no increase in tone in response to either caffeine (1mM) or to noradrenaline (1μM) indicating that they were devoid of functional intracellular calcium stores. They were, however, responsive to changes in the free calcium ion concentration. Of the 18 tissues that were skinned 8 were responsive to changes in free Ca^{2+}, the remainder were discarded. Tension was maximal at ∿5μM Ca^{2+}. The EC50 for Ca^{2+} in control strips was 159 ± 23 nM (mean ± sem), (n=5). In the presence of adenosine (300μM) the EC50 was 141 ± 29 nM (n=5) which was not significantly different from the control value. Similarly, the maximum tension attained was 83 ± 11 mg (n=5) in control strips and 79 ± 10 mg (n=5) in the presence of adenosine (not significantly different at $p<0.05$). It is of interest, however, that both cAMP (2μM) and cGMP (2μM) reduced the maximal tension of skinned fibres to approximately 40% of control values in the 3 preparations on which these compounds were used.

Although adenosine does not by itself affect the sensitivity of the contractile apparatus to Ca^{2+} the question is now posed whether adenosine induces relaxation in the intact cell via the production of an intermediary which then regulates the contractility of the tissue without necessarily diminishing intracellular free calcium.

Supported by the British Heart Foundation.

Drury, A.N. and Szent-Gyorgyi, A. (1929) J. Physiol. 68, 213-237.
Long, C.J. and Stone, T.W. (1986) Br. J. Pharmac. 87, 8P.
Ruegg, J.C. et al. (1983) Basic Res. Cardiol. 78, 462-471.

DOPAMINERGIC CONTROL OF TYROSINASE ACTIVITY IN HAIR FOLLICULAR MELANOCYTES OF THE C_3H-HeA^{*vy} MOUSE.

Susan A. Burchill & A.J. Thody (Introduced by Dr. F. Williams), Department of Dermatology, University of Newcastle upon Tyne.

α-MSH increases eumelanin synthesis in the hair follicular melanocytes of the C_3H-HeA^{*vy} mouse through its effect on tyrosinase, a key enzyme in the melanin pathway. Conversely bromocriptine, a dopamine agonist that blocks α-MSH secretion, inhibits eumelanin synthesis in this mouse but this effect is not related to a decrease in circulating α-MSH (Burchill & Thody, 1985). In this study we have examined the possibility that dopaminergic mechanisms act directly to inhibit hair follicular melanocytes in the C_3H-HeA^{*vy} mouse.

Hair growth was initiated by plucking in C_3H-HeA^{*vy} mice and skin explants taken 8 days later for in vitro incubation. Skin explants were incubated in HEPES buffered RPMI medium at 37°C in the presence or absence of bromoriptine for up to 24h. Bromocriptine decreased the level of tyrosinase activity in the skin explants at all times studied. This inhibition of tyrosinase activity was blocked when the dopamine antagonists haloperidol and spiperone were included in the incubation medium. The specific D_2-agonist LY 171555 also decreased tyrosinase activity in skin explants in a dose related manner. This inhibitory effect was blocked by sulpiride. The D_1-agonist SKF 38393 had no effect on tyrosinase activity.

We conclude that dopamine agonists have a direct inhibitory effect on hair follicular melanocytes in the C_3H-HeA^{*vy} mouse through a D_2 receptor mechanism. These results suggest that the hair follicular melanocytes of this mouse are not regulated solely by α-MSH and that dopaminergic mechanisms may also be important.

Burchill SA & Thody AJ (1985) Effect of bromocriptine on tyrosinase activity and coat colour in the mouse. Journal of Investigative Dermatology, 84: 443.

α-METHYLDOPA DOSE DEPENDENTLY LOWERS BLOOD PRESSURE: EFFECT OF THE OPIATE RECEPTOR ANTAGONIST NALTREXONE.

W. de Jong, P.L.M. van Giersbergen* & V.M. Wiegant, Rudolf Magnus Institute for Pharmacology, Medical Faculty, University of Utrecht, Vondellaan 6, 3521 GD Utrecht, The Netherlands.

In spontaneously hypertensive rats it was shown that the hypotension and brady - cardia induced by clonidine, an α_2-adrenoceptor agonist, could be blocked or reversed by the opiate receptor antagonist naloxone (Farsang & Kunos, 1979). However, the effect of naloxone on the fall in BP produced by either clonidine or α-methyldopa is controversial in normotensive animals (Bennet et al, 1982, Brown et al, 1984). In the present study we investigated the possible involvement of endogenous opioid systems in the mechanism of action of α-methyldopa.

Normotensive, conscious, unrestrained male Wistar rats were used. The cisterna magna was cannulated under Hypnorm anaesthesia for central administration of drugs. Six days later the femoral artery was catheterized using ether anaesthesia to enable continuous registration of BP. After another two days the experiments were performed.

α-methyldopa dose dependently lowered BP of the animals reaching a maximum 4 hrs after administration (table 1). Depending on the dose of α-methyldopa employed, pretreatment with naltrexone (125 µg/kg intracisternally [i.c.]) 20 min prior to α-methyldopa significantly inhibited this effect of α-methyldopa (table 1). In addition, injection of 0.5 mg/kg of α-methyldopa resulted in a decrease in BP by 28.3 ± 1.2 mmHg. This fall in BP was partially inhibited by doses of 25 µg/Kg (-21.5 ± 1.5 mmHg) and 125 µg/kg (-15.3 ± 2.6 mmHg) of naltrexone, but almost completely blocked by a dose of 725 µg/kg of naltrexone (-7.8 ± 3.1 mmHg). Naltrexone alone in doses of 125 and 725 µg/kg i.c. did not affect BP

Table 1. Effect of naltrexone (125 µg/kg i.c.) on the influence of increasing doses of α-methyldopa on mean arterial pressure (MAP).

dose of α-methyldopa	pretreatment	n	base line value for BP (mmHg)	MAP after 4 hrs of registration	% inhibition by naltrexone
0.25 mg/kg	saline	10	122 ± 2.5	-15.8 ± 2.3	
	naltrexone	8	113 ± 3.9	-2.8 ± 2.5***	82.3
0.375 mg/kg	saline	8	112 ± 2.8	-21.5 ± 2.5	
	naltrexone	6	111 ± 3.8	-7.2 ± 2.7***	66.5
0.50 mg/kg	saline	9	115 ± 3.0	-28.3 ± 1.2	
	naltrexone	6	112 ± 3.1	-15.3 ± 2.6***	45.9
1.00 mg/kg	saline	6	115 ± 3.6	-30.2 ± 2.0	
	naltrexone	6	116 ± 5.2	-23.3 ± 2.3*	22.8

Values are expressed as mean ± s.e. * : $0.05 < p \leq 0.01$ *** $p < 0.001$ as compared to saline pretreated controls.

These results indicate that the endogenous opioids play a role in the central hypotensive mechanism of action of α-methyldopa. However, the nature of the involved opioid peptides is still unknown as are site and exact mechanism of this interaction. Prelimanary results suggest an involvement of endorphins.

Farsang, C. & Kunos, G. (1979) Br. J. Pharmac. 67, 161-164.
Bennet, D.A., DeTeo, J.J., Elko, E.E. & Lal, H. (1982) Drug Dev. Res. 2, 175-179.
Brown, M.J., Conway, E.L. & Dollery, C.T. (1984) J. Pharmacol. Exp. Ther. 229, 803-808.

THE SELECTIVITY OF THE PARTIAL AGONIST ACTIVITY OF XAMOTEROL IN MAN MEASURED BY ITS EFFECTS IN THE PRESENCE AND ABSENCE OF ICI 118,551.

P.M. McCaffrey, J.G. Riddell and R.G. Shanks, Department of Therapeutics and Pharmacology, The Queen's University of Belfast, Belfast, Northern Ireland

Xamoterol is a new cardioselective beta-adrenoceptor partial agonist (Nuttall & Snow, 1982). The selectivity of the actions of xamoterol, salbutamol and prenalterol was assessed in man by comparing the effects of these drugs and placebo on heart rate, blood pressure, forearm blood flow, finger tremor and exercise heart rate in the presence and absence of the specific $beta_2$-adrenoceptor antagonist ICI 118,551 (Bilski et al., 1983).

Eight healthy male volunteers received double blind, at weekly intervals, in a randomised Latin Square design single oral doses of salbutamol 8 mg (Sa), prenalterol 50 mg (Pr), xamoterol 200 mg (Xa), salbutamol 8 mg with ICI 118,551 25 mg (Sa+), prenalterol 50 mg with ICI 118,551 25 mg (Pr+), xamoterol 200 mg with ICI 118,551 25 mg (Xa+), ICI 118,551 25 mg (ICI) and placebo.

After 30 minutes of supine rest and before taking the treatment, heart rate, blood pressure, forearm blood flow and finger tremor were measured. After treatment the subjects remained supine and the measurements were repeated at 30 minute intervals for 3 hours, when an exercise step test (46 cm step, 32 steps per min) was performed. Exercise heart rate (EHR) was measured within 5 seconds of completing exercise. Heart rate (HR) was measured from a direct writing ECG, systolic and diastolic blood pressure (SBP, DBP) using a Hawksley random zero sphygmomanometer, forearm blood flow (FBF) using venous occlusion plethysmography, and finger tremor (FT) using an accelerometer attached to the dorsum of the middle finger of the left hand. SPSS was used for the analysis of variance.

The table shows HR, SBP, DBP, %ΔFBF, %ΔFT and EHR 3 hours after treatment ($\bar{X}$ ± sem)

Drug	HR	SBP	DBP	%ΔFBF	%ΔFT	EHR
Pl	59 ± 3	103 ± 2	64 ± 3	7 ± 7	139 ± 121	181 ± 3
Xa	65 ± 3	124 ± 5*	64 ± 3	21 ± 8	83 ± 41	158 ± 4*
Xa+	60 ± 3	124 ± 5*	66 ± 3	26 ± 14	39 ± 27	156 ± 4*
Sa	86 ± 7*	110 ± 5*	54 ± 3*	62 ± 20*	3598 ± 1376†	187 ± 4*
Sa+	59 ± 2	108 ± 4	67 ± 3	-4 ± 10	69 ± 58	174 ± 6
Pr	82 ± 5*	130 ± 4*	59 ± 5	46 ± 14*	348 ± 88†	177 ± 5
Pr+	77 ± 4*	128 ± 5*	64 ± 3	19 ± 8	138 ± 51	174 ± 5
ICI	57 ± 2	104 ± 3	65 ± 3	-7 ± 6	2 ± 20	175 ± 5

*P<0.01 v placebo; †P<0.02 v placebo

Supine heart rate was increased by salbutamol, prenalterol and prenalterol with ICI 118,551. Systolic blood pressure was increased by xamoterol, xamoterol with ICI 118,551, salbutamol, prenalterol and prenalterol with ICI 118,551. Salbutamol reduced diastolic blood pressure. Prenalterol and salbutamol increased both forearm blood flow and finger tremor. Xamoterol and xamoterol with ICI 118,551 reduced exercise heart rate. Salbutamol increased exercise heart rate. There was no difference in the results obtained for xamoterol and xamoterol with ICI 118,551. Prenalterol caused a greater increase in forearm blood flow than prenalterol with ICI 118,551. Diastolic blood pressure was lower and forearm blood flow and finger tremor higher on salbutamol than on salbutamol with ICI 118,551. These results indicate that the partial agonist activity of xamoterol is selective for the $beta_1$ receptor, that salbutamol is a selective $beta_2$-agonist and that prenalterol acts at both receptors.

Nuttall, A. & Snow, H.M. (1982) Br. J. Pharmac., 77, 381-388.
Bilski, A.J. et al. (1983) J. Cardiovas. Pharmac., 5, 430-437.

IN VITRO DETERMINATION OF β-AGONISTIC PROPERTIES OF TULOBUTEROL, A NEW β_2-SYMPATHICOMIMETIC DRUG.

J.A.M. Raaijmakers and G.K. Terpstra.
Department of Pulmonary Diseases, State University Hospital Utrecht, Catharijnesingel 101, 3511 GV Utrecht, The Netherlands.

Radioligand binding studies were developed to characterize pharmacological receptors in vitro. Using this type of assay, the number and dissociation constant (K_D) can be studied as well as the interaction of agonists with the receptor. The latter can be achieved by performing agonist inhibition of radiolabelled antagonist binding. This method makes it possible to analyse the relative affinity of hormones or mimetic drugs to a certain receptor and above all, compare the potencies of different drugs with regard to their interaction with the receptor.

We have used the above mentioned method to compare a new β_2-mimetic drug tulobuterol (1-(0-chlorophenyl)-2-butylaminoethanol hydrochloride) with terbutaline and salbutamol, well established β_2-mimetic drugs.

In rat the inhibition of ^{3}H-dihydroalprenolol binding with the drugs was best fit in a two binding sites model, showing high and low affinity binding sites. The high affinity sites of terbutaline and tulobuterol had similar K_D values ($1.6 \pm 0.8 \times 10^{-7}$M and $1.5 \pm 0.4 \times 10^{-7}$M resp.). However 64.6% of the total binding was inhibited with high affinity by tulobuterol while 21.9% was inhibited with high affinity by terbutaline. Salbutamols high affinity site displayed a 5 times lower affinity (K_D 0.9×10^{-6} M) and 61.7% of the sites were high affinity sites. Experiments with guanine nucleotides showed that the tulobuterol-receptor complex was more potent to couple to the adenylate cyclase than the terbutaline-, and salbutamol-receptor complex. Furthermore, the inhibition of ^{3}H-DHA binding by tulobuterol was more pronounced in lung tissue than in cardiac tissue, indicating the β_2-selectivity of the drug. When human lung membranes of normal subjects were used, similar potencies of the drugs tested were found.

These results indicate that tulobuterol is a selective β_2-mimetic drug that seems more potent than salbutamol and terbutaline in terms of binding to the β-adrenoceptor and coupling of the drug-receptor complex to the adenylate cyclase.

THE ROLE OF THE cAMP-DEPENDENT PROTEIN KINASE IN PHOSPHORYLATION OF THE β-ADRENOCEPTOR.

D. Cooney and A.K. Keenan, Department of Pharmacology, University College Dublin.

Desensitization of catecholamin-stimulated adenylate cyclase activity is demonstrable in membranes derived from turkey erythrocytes pretreated with isoprenaline (Hoffmann et al., 1980). Although no change in β-adrenoceptor number was detectable after isoprenaline treatment, the receptor peptides were shown to be of larger apparent molecular weight, indicating that structural and/or conformational alterations had occurred during the desensitization process (Nambi et al., 1984). The effects of isoprenaline can be mimicked by cAMP treatment of erythrocytes and desensitization produced in this way has been shown to be highly correlated with incorporation of phosphate into the receptor in vivo (Stadel et al., 1983). In the present study we attempted to phosphorylate, in vitro, the purified β_1-receptor isolated from turkey erythrocyte membranes using a purified cAMP-dependent protein kinase (A-kinase).

The β-receptor was solubilized in digitonin or lauroyl sucrose and purified 5,000 fold by Sepharose-alprenolol affinity chromatography. Receptor-containing liposomes were prepared using SM-2 Biobeads. The C-subunit and the RII regulatory subunit of the A-kinase were purified from bovine and rat heart, respectively. Purified receptor (0.2-2.0 pmol) was incubated in a total volume of 200 µl with 0.1-0.3 µg of the catalytic subunit of the A-kinase (2 units/min x mg) and 5 µM [γ-^{32}P]-ATP (specific activity 3 Ci/mmol) in a buffer containing 90 mM NaCl, 10 mM Tris, 10 mM $MgCl_2$, 1 mM mercaptoethanol, 1 µM cAMP, 0.02% digitonin, 0.1 mg/ml BSA (pH 7.4). Following incubation at 30°C for 15 min, 50 µl SDS sample buffer (containing 5 mM ATP) was added. (RII phosphorylation was also carried out under the above conditions). Samples were run on 11% SDS-PAGE gels (Laemmli, 1970) which were subsequently subjected to autoradiography. Receptor proteins were visualized with the photoaffinity label [^{125}I]-cyanopindololazide.

Incubation of the purified receptor with C-subunit resulted in strong phosphorylation of a 50 KD protein. This protein was shown not to be the β-receptor since (a) it could not be specifically eluted from an affinity column and (b) it was insensitive to proteolytic degradations characteristic of the receptor. Compared to RII, the highly purified receptor was a poor substrate for phosphorylation by the A-kinase. Incorporation of phosphate into the receptor was not improved by (i) increasing the ratio of kinase to receptor, (ii) detergent removal and receptor incorporation into liposomes prior to phosphorylation, (iii) receptor preincubation with the activating ligands isoprenaline + Gpp(NH)p.

We conclude that the turkey erythrocyte β-adrenergic receptor purified in a soluble or liposome-reconstituted form is a poor substrate for phosphorylation by the A-kinase. We propose that although this kinase has a role in vivo, it may only be the first of a number of kinases involved in a cascade that eventually leads to receptor phosphorylation.

This work was carried out at the University of Würzburg, during the tenure of a DAAD fellowship (by D.C.). The support of the SFB 176 and gifts of A-kinase components (S. Lohmann and U. Walter) are acknowledged.

Hoffmann, B.B. et al. (1980) J. Cycl. Nucleotide Res. 5, 355-366.
Laemmli, U.K. (1970) Nature (Lond) 227, 680-685.
Nambi, P. et al. (1984) J. Biol. Chem. 259, 4629-4633.
Stadel, J.M. et al. (1983) Proc. Natl. Acad.Sci. U.S.A. 80, 3173-3177.

PROTECTIVE ACTIVITY OF R 58735 UPON OUABAIN INTOXICATION IN ANAESTHETIZED GUINEA-PIGS.

H.W.G.M. Boddeke, J.B. Heynis, F.A.M. Jonkman and P.A. van Zwieten, Division of Pharmacotherapy, University of Amsterdam, Plantage Muidergracht 24, 1018 TV Amsterdam, The Netherlands.

It has been shown by several authors that calcium entry blockers possess protective properties upon ouabain-induced arrhythmias. Jonkman et al. (1986) showed, that calcium entry blockers delay the onset of cardiac arrhythmia in anaesthetized guinea-pigs, probably by preventing a situation of calcium overload of myocardial cells. In the present study the effect on left ventricular pressure, heart rate and coronary flow of the new calcium entry blocker R 58735 (4-[(2-benzothiazolyl) methylamino]-α-[(4-fluorophenoxy)methyl]-1-piperidine ethanol monohydrochloride) have been investigated in the Langendorff preparation of the guinea-pig heart. The hearts were perfused with Tyrode solution at 37° C and at a calcium concentration of 1.3 mM. The left ventricular pressure was measured by means of an intraventricular balloon, filled with saline. In order to prevent glass wall adsorption, R 58735 was infused via a teflon catheter into the perfusate, directly before the heart. Moreover, the protective effect of R 58735 upon ouabain-induced arrhythmias was studied in urethane (1.5 g/kg i.p.) anaesthetized guinea-pigs.

In the Langendorff preparation the heart rate fell from 158 to 79 b/min (49.8 ± 3.1 %). In the paced hearts (frequency 4 Hz) the contractile activity was completely abolished and the coronary flow increased from 9.6 to 14.4 ml/min (48.8 ± 4.4 %) at a concentration of 3.10^{-6} M R 58735 ($p < 0.05$, n=6).

Pretreatment with R 58735 (1 mg/kg) via the carotid artery significantly postponed the occurrence of toxic ECG changes upon ouabain infusion (4 µg/min i.v.). Ventricular fibrillation was observed after 20.1 ± 2.4 min in the control animals, but it occurred after 30.6 ± 1.6 min ($p < 0.05$, n=6) in the R 58735 treated animals. In conclusion, R 58735 is a new calcium entry blocker with a weak cardiodepressant activity and a potent protective effect upon ouabain-induced arrhythmias in guinea-pigs.

F.A.M. Jonkman, H.W.G.M. Boddeke and P.A. van Zwieten. Protective activity of calcium entry blockers against ouabain intoxication in anaesthetized guinea-pigs. J. Cardiovasc. Pharmacol. 1986 (in press).

DECREASED IN VIVO AND IN VITRO RESPONSES TO VASOCONSTRICTOR AGENTS IN PREGNANT NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RATS.

G. Massicotte, A. Parent, J. Saint-Louis & E.L. Schiffrin, Experimental Hypertension Laboratory, Clinical Research Institute of Montreal, Montreal, Quebec, Canada H2W 1R7

Pregnancy in human and rat is normally associated to a substantial decrease in blood pressure (BP). Similar observation has been made in spontaneously hypertensive rats (SHR) (St-Louis and Massicotte, 1985). Although the timing of this decrease in BP is different in these two normotensive species, it is generally attributed to a decrease in vascular responsiveness (VR) to angiotensin II (AII). The purpose of the present study is to ascertain the contribution of the VR to vasoconstrictor agents in this decrease in BP in pregnant normotensive and hypertensive rats.

We have measured the concentration-response (C-R) curves in vivo to AII and vasopressin (AVP) in conscious pregnant (9th and 20th days) and cycling normotensive rats (Sprague-Dawley (SD), and Wistar-Kyoto (WKY)) and SHR. We have also investigated the in vitro effects of AVP and norepinephrine (NE) in perfused mesenteric artery and to AII and NE in the isolated portal vein in similar rats.

In vivo experiments were conducted as previously reported (St-Louis and Regoli, 1973) in conscious catheterized and unrestrained rats. Vasoconstrictor agents were given in bolus injection (50 µl) via the jugular vein catheter and the BP was measured from the carotid artery catheter with a Statham P-23 ID pressure transducer and recorded on a Grass polygraph. In vitro perfusion of the superior mesenteric artery was performed as previously described (St-Louis and Schiffrin, 1984), while isolated longitudinal portal vein strips were prepared as described by Couture et al. (1978).

At the end of gestation (20th day), the C-R curves to AII and AVP for the increase in BP was shifted to the right by a factor varying from 1.3 to 2.1 in the normotensive and SHR; no consistent change was observed at the 9th day of gestation. On the perfused mesenteric artery from rats at the 20th day of gestation, the C-R curves for the increase of perfusion pressure by AVP and NE were shifted to the right by comparison to non-pregnant female rats. The increase in EC_{50} to both agents in pregnant rats was around two-fold while no consistent effect was observed on the maximum response. On isolated veins, similar results were obtained to AII and NE except that the increase in EC_{50} in pregnant rats was smaller in magnitude but still significant.

These data show that the in vivo and in vitro decreased responsiveness to vasoconstrictor agents in pregnant rats is similar in normotensive and hypertensive animals. This suggests that the factor(s) responsible for this refractoriness effect is a general mechanism affecting vascular smooth muscle of both arteries and veins, and that this decreased responsiveness might be the mechanism by which the blood pressure is decreased during gestation in the rat models used in this study.

Couture, R., F. Rioux & D. Regoli (1978) Clin. Exp. Hypertension 1, 393
St-Louis, J. & G. Massicotte (1985) Life Sci. 37, 1351
St-Louis, J. & D. Regoli (1973) Rev. Can. Biol. 32, 81
St-Louis, J. & E.L. Schiffrin (1984) Life Sci. 35, 1489

Supported by Medical Research Council of Canada and Quebec Heart Foundation.

BLOOD PRESSURE LOWERING EFFECT OF CENTRALLY AND PERIPHERALLY ADMINISTERED APOMORPHINE IN ANAESTHETIZED RATS.

A.G. Dupont, P. Van der Niepen, L. Vanhaelst, Department of Pharmacology, University of Brussels (VUB), Brussels, Belgium.

Evidence has been presented that central dopamine receptors might be involved in the hypotensive action of pergolide, both in rats (Jadhav *et al.*, 1983) and in dogs (Barrett and Lokhandwala, 1983). By contrast, Bogaert *et al.* (1978) have shown that the hypotension produced by intravenous apomorphine in the dog cannot be explained by a central mechanism. We therefore studied the blood pressure lowering effects of centrally and peripherally administered apomorphine in anesthetized rats and compared them to the effects of clonidine.

The experiments were done in normotensive Wistar rats weighing 230-320 g, anesthetized with pentobarbital. A cannula was inserted into the carotid artery for direct measurement of arterial blood pressure. The jugular vein was cannulated for intravenous administration of drugs. In order to administer drugs within the central nervous system, rats were placed in a stereotaxic apparatus (SR-6, Narishige, Tokyo) and a stainless steel needle was inserted into the third ventricle through which drugs were administered in a volume of 10 µl/kg.

Table 1 Decrease of mean blood pressure produced by apomorphine and by clonidine following intracerebroventricular (ICV) and intravenous (IV) administration (mean ± SEM; *p < 0.05 as compared to ICV; Wilcoxon test).

	ICV	IV
apomorphine 3 µg/kg	8.0 ± 0.7%	16.0 ± 1.1%*
apomorphine 10 µg/kg	16.2 ± 4.7%	17.7 ± 5.8%
clonidine 10 µg/kg	36.4 ± 3.4%	22.5 ± 2.8%*

In a first series of experiments apomorphine 3 µg/kg and 10 µg/kg, and clonidine 10 µg/kg were administered ICV and IV in random order (n = 6 for each dose). Apomorphine 3 µg/kg IV produced an immediate reduction of blood pressure; when this same dose was administered ICV the blood pressure fall was slower in onset and of smaller magnitude. The hypotensive effect of ICV administration of 10 µg/kg apomorphine was of similar magnitude as, but slower in onset than after IV administration. IV administration of clonidine produced an initial increase, followed by a decrease of blood pressure; ICV administration of clonidine produced an immediate and larger fall in blood pressure (Table 1).

In a second series of experiments the influence of IV administration of domperidone (10 µg/kg) on the hypotensive effect of ICV and IV administered apomorphine (10 µg/kg) was studied; the dopamine receptor antagonist domperidone does not readily cross the blood-brain barrier (Laduron and Leysen, 1979). The reproducibility of the effects of ICV and IV apomorphine in the absence of antagonist was confirmed in separate groups of rats. Apomorphine 10 µg/kg IV reduced blood pressure by 27.5 ± 4.3% before and by 8.3 ± 2.4% after IV domperidone (n = 6; p < 0.05). Apomorphine 10 µg/kg ICV reduced blood pressure by 18.7 ± 2.5% before and by 4.3 ± 2.6% after IV domperidone (n = 6; p < 0.05).

The results of this study confirm that the hypotensive effect of clonidine is centrally mediated, but suggest that the hypotensive effect of apomorphine in rats is mainly due to stimulation of peripheral dopamine receptors.

Barrett, R.J. & Lokhandwala, M.F. (1983) Eur. J. Pharmacol. **96**, 211-216.
Bogaert, M.G. *et al.* (1978) Br. J. Pharmacol. **63**, 481-484.
Jadhav, A.L. *et al.* (1983) Naunyn-Schmiedeberg's Arch. Pharmacol. **324**, 281-286.
Laduron, P.M. & Leysen, J.E. (1979) Biochem. Pharmacol. **28**, 2161-2165.

600P

SELECTIVE INHIBITION OF HIGH AND LOW AFFINITY INOTROPIC COMPONENTS OF OUABAIN BY AMILORIDE DERIVATIVES.

M. Finet & T. Godfraind, Laboratoire de Pharmacodynamie Générale et de Pharmacologie, Université Catholique de Louvain, UCL 73.50, av. E. Mounier 73, B-1200 Bruxelles (Belgium).

The inotropic dose-effect curve of ouabain in rat ventricles is biphasic and is believed to be due to the interaction of the glycoside with high and low affinity binding sites (Finet et al., 1983; Grupp et al., 1985). Amiloride has been reported to inhibit the Na^+/H^+ and the Na^+/Ca^{++} exchanges in cardiac cells. A dissociation of these effects has been shown for the derivative compounds ethylisopropylamiloride (EIPA) and dichlorobenzamil (DCB) : EIPA seems to be principally an inhibitor of the Na^+/H^+ exchange and DCB an inhibitor of the Na^+/Ca^{++} exchange (Frelin et al., 1984).
In the present experiments, we have studied the biphasic positive inotropic effect of ouabain in rat ventricles in the presence of EIPA and DCB.

Right ventricular strips were electrically stimulated (1 Hz) in a Tyrode solution containing 6 mM KCl (30°C), under an initial resting tension of 500 mg.

EIPA (10-20 uM) produced a negative inotropic effect (8±2 %, n=6 and 22±5 %, n=6). At these concentrations the effect evoked by ouabain was depressed in a concentration-dependent manner. An analysis (non linear regression) of the experimental data assuming two saturable components showed that EIPA 10 uM depressed and EIPA 20 uM abolished the high affinity component of ouabain inotropic effect whereas it did not significantly modify the low affinity inotropic effect related to high concentrations of ouabain. At the EIPA concentrations studied, there was no observable modification of the inotropic effect of Bay k 8644 (10 nM, 0.3 uM), isoprenaline (10 nM, 1 uM) or low extracellular potassium (2.5 mM).
DCB (10-20 uM) evoked a weak positive inotropic effect (less than 10 %) whereas DCB 40 uM produced a negative inotropic effect that reached a 20±3 % (n=6) diminution of the contractile force after 50 min. and that led to a contraction arrest after 120 min. DCB (10-20 uM) depressed dose-dependently the positive inotropic effect of ouabain. This depression was much more pronounced on the low affinity component of ouabain than on the high affinity inotropic component. DCB (10-20 uM) did not significantly modify the inotropic effect of Bay k 8644 (10 nM, 0.3 uM) and isoprenaline (10 nM, 1 uM).

These results show that the inotropic effect of ouabain related to its high affinity sites is selectively sensitive to the Na^+/H^+ exchange inhibitor EIPA, whereas its effect related to the low affinity sites is especially sensitive to the Na^+/Ca^{++} exchange inhibitor DCB.

M. Finet is Aspirant F.N.R.S.

Finet, M., Godfraind, T. & Noel, F. (1983) Br. J. Pharmac., 80, 751-759.
Frelin, C., Vigne, P. & Lazdunsky, M. (1984) J. Biol. Chem., 259, 8880-8885.
Grupp, I., Im, W.-B., Lee, C.O., Lee, S.-W., Pecker, M.S. & Schwartz, A. (1985) J. Physiol., 360, 149-160.

CONTRACTION AND RELAXATION OF MICROVESSELS ISOLATED FROM RAT BRAIN.

T. Godfraind & Nicole Morel, Laboratoire de Pharmacodynamie Générale et de Pharmacologie, Université Catholique de Louvain, UCL 7350, avenue Emmanuel Mounier 73 - B1200 Bruxelles, Belgium.

Calcium entry blockers are used to treat migraine which could be due to spastic alterations of the cerebral circulation (Towart, 1984). The aim of this work was to perform direct examination of the contractile behaviour of cerebral microvessels and of its sensitivity to the Ca entry blocker flunarizine. Contractile properties of cerebral microvessels have been tested by investigating the change of their diameter in response to KCl-evoked depolarization.

Cerebral microvessels were isolated from the gray matter of rat brain by mild disruption of the tissue and filtration on nylon sieves (Morel and Godfraind, 1985). The preparation consisted in multibranching capillaries with segments of small arteries. Segments of vessels, with a length of approximatively 500 um and a diameter in the range 20-60 um were cannulated using glass pipettes and immerged in thermostatized organ bath containing oxygenated Krebs physiological solution. They were examined with a microscope equipped with a camera. Vessel diameter was measured on photographic recordings.

When the physiological solution was changed to a solution containing 100 mM KCl, the vessel diameter was reduced by 50 to 90 %. The contraction was sustained for periods as long as one hour, it was reversed by returning to the normal physiological solution and it could be reproduced by the readmission of the depolarizing solution to the organ chamber. Relaxation of cerebral microvessels was also observed when the Ca entry blocking drug flunarizine was added to the KCl-depolarizing solution to reach the final concentration of 10^{-6} M. The time course of this relaxation was consistent with that observed in small resistant vessels.

These data indicate that microvessels isolated from rat brain maintain functional voltage-operated Ca channels. They can be considered as a useful tool in the study of cerebral microcirculation.

Nicole Morel is I.R.S.I.A. fellow.

Morel, N. & Godfraind, T. (1985) Arch. Int. Pharmacodyn. Ther., 277, 332.

Towart, R. (1984) In : "Calcium entry blockers in cardiovascular and cerebral dysfunctions" Godfraind T., Herman A.G. & Wellens D. (eds.), Martinus Nijhof Publishers, The Hague, p. 263-270.

602P

POSSIBLE CONTRIBUTION OF LIGNANS TO THE MAMMALIAN DIGITALIS-LIKE ACTIVITY.

Mireille Fagoo & T. Godfraind, Laboratoire de Pharmacodynamie Générale et de Pharmacologie, Université Catholique de Louvain, UCL 73.50, av. E. Mounier 73, B-1200 Bruxelles.

Several attempts have been made to identify an endogenous digitalis-like regulator of the sodium pump which could be involved in hypertension (de Wardener & Clarkson, 1985; Castaneda-Hernandez & Godfraind, 1984). Mammalian lignans have been discovered in 1980 but their biological role is still unknown (Setchell et al., 1980). Among them, enterolactone shares with cardiac glycosides a gamma-butyrolactone ring; in the 10^{-4} M range, it inhibits specifically Na^+,K^+-ATPase and displaces ouabain from its binding sites (Fagoo et al., 1986).

The aim of the present work was to study the potential contribution of this lignan in the endogenous digitalis-like activity. We have therefore examined the chromatographic behaviour of enterolactone, comparatively to that of cardiodigin, endogenous digitalis-like factor found in every mammalian tissue so far studied (Godfraind et al., 1982; Fagoo & Godfraind, 1985). Cardiodigin was purified from beef adrenal, heart, hypophysis and hypothalamus water homogenates as previously reported (Fagoo & Godfraind, 1985). The procedure comprises successively : protein precipitation by methanol, lipids extraction by cyclohexane and reverse-phase low pressure liquid chromatography. Cardiodigin was characterized by its ability to compete with ouabain binding to Na^+,K^+-ATPase preparations.

The present chromatographic analyses were performed with silicagel thin-layer plates, using two elutions systems. The first one consisted in chloroform-methanol-ammoniac (65:35:5) (I) and the second, was the same system in the proportions (300:15:5) (II). Aldosterone and corticosterone were used as external standards. The active digitalis-like fraction was scraped from an area comprised between a Rf of 0.7 ± 0.05 (mean±S.E.) (n=5) to 0.84 ± 0.04 in system I and between 0 to 0.05 ± 0.03 in system II. The Rf's of enterolactone were 0.68 ± 0.06 (n=5) (I) / 0.09 ± 0.03 (n=4) (II). The migration properties of the digitalis-like fraction extracted from mammalian tissues are therefore very similar to those of enterolactone.

Mammalian lignans show physicochemical properties similar to those of steroids. The present study further indicates that such lignans whose daily excretion is comparable to that of many steroid metabolites could represent at least part of the bulk of digitalis-like activity extractable from mammalian tissues.

Mireille Fagoo is Chargé de Recherches F.N.R.S.

Castaneda-Hernandez, G. & Godfraind, T. (1984) Clin. Sci., 66, 225-228.
de Wardener, H.E. & Clarkson, E.M. (1985) Physiol. Rev., 65, 658-759.
Fagoo, M., Braquet, P., Robin, J.P., Esanu, A. & Godfraind, T. (1986) Biochim. Biophys. Res. Commun., 134, 1064-1070.
Fagoo, M. & Godfraind, T. (1985) FEBS Lett., 184, 150-154.
Godfraind, T., De Pover, A., Castaneda-Hernandez, G. & Fagoo, M. (1982) Arch. Int. Pharmacodyn. Ther., 258, 165-167.
Setchell, K.D.R., Lawson, A.M., Mitchell, F.L., Adlercreutz, H., Kuk, D.N. & Axelson, M. (1980) Nature, 287, 740-742.

AN INVESTIGATION OF AGE-RELATED CHANGES OF β-ADRENOCEPTOR MEDIATED RESPONSIVENESS IN THE RAT.

James R. Docherty, Department of Clinical Pharmacology, Royal College of Surgeons in Ireland, Dublin 2, Ireland.

There are many reports of a reduced tachycardia to the beta-agonist isoprenaline in the elderly (Vestal et al., 1979),or of a reduced beta-adrenoceptor mediated vasodilation in isolated blood vessels from aged rats (O'Donnell and Wanstall, 1984), or a reduced response of adenylate cyclase to beta-adrenoceptor agonists with increasing age (see Kelly and O'Malley, 1984). We have chosen to examine the cardioaccelerator and vasodepressor responses to isoprenaline in 4 age groups of anaesthetised and pithed rats.

Male Sprague-Dawley rats of 4 age groups (1.5, 3, 6, and 24 months) were anaesthetised or pithed under pentobarbitone anaesthesia, and cannulae were placed in the carotid artery and jugular vein. The pithing rod was used as an electrode to stimulate the cardioaccelerator nerves with a single stimulus. Isoprenaline was administered cumulatively in 0.5 log unit increments.

In anaesthetised rats, there was no significant difference between aged (24 month) and adult (3 & 6 month) in the potency of isoprenaline at lowering diastolic blood pressure, although isoprenaline was less potent in juvenile (1.5 month) animals. There was no significant differences between age groups in the cardioacceleration to isoprenaline in anaesthetised animals, although isoprenaline was significantly less potent in 24 month old than in 3-6 months combined.

In pithed animals, there was no significant difference between groups in the cardioaccelerator potency of isoprenaline, nor was there a difference between 24 month and 3-6 month combined. There was no significant age-related alteration in the cardioacceleration to a single stimulus, although the response in 6 month, but not in 3 month, was significantly greater than in 24 month.

Overall, this study lends no support for a general reduction in beta-adrenoceptor mediated responsiveness with ageing. Differences in the tachycardia to isoprenaline seen in man may reflect reduced baroreflex compensation to falls in DBP produced by isoprenaline, since baroreflex function is reported to be reduced in the aged.(Docherty et al., 1986).

Docherty, J.R. et al.,(1986) J. Cardiovasc. Pharmacol. (in press).
Kelly,J. & O'Malley, K. (1984) Clin. Sci.66, 509-515.
O'Donnell,S. & Wanstall, J. (1984). J. Pharmacol. Exp. Ther. 228,733-738.
Vestal, R. et al. (1979). Clin. Pharmacol. Ther. 26, 181-186.

Supported by the Medical Research Council of Ireland and by the Royal College of Surgeons in Ireland.

HEMODYNAMIC EFFECTS OF THE CARDIOTONIC AGENT OPC-8212 IN DOGS.

D. Ruhl, S. Schollengerger, J.C.A. van Meel and E. Wolfram, Dept. of Cardiovascular Research, Dr Karl Thomae GmbH, 7950 Biberach/Riss, FRG.

OPC-8212 (3,4-dihydro-6- 4-(3,4-dimethoxybenzoyl)-1-piperazinyl-2-1H)-quinolinone) is a new positive inotropic agent. Interesting properties of this substance are the relatively weak effects on arterial blood pressure and heart rate and the long duration of action (for review see Arzneim.Forsch/Drug Res.34, 333-402, 1984). We investigated the hemodynamic effects of OPC-8212 in anaesthetized dogs and conscious chronically instrumented dogs. OPC-8212 was infused at 30, 100 and 300 µg/kg/min (30 min for each infusion) into chloralose-urethane-pentobarbitone anaesthetized open-chest dogs. Data represent mean values ± SEM (n=6).

Left ventricular dp/dtmax, cardiac output and stroke volume index increased from 2829 ± 248 to 5642 ± 843 mm Hg/s, 3.51 ± 0.27 l/min to 4.26 ± 0.29 l/min and 0.88 ± 0.10 to 1.10 ± 0.07 ml/kg, respectively. Heart rate decreased (from 157 ± 7 to 141 ± 9 bpm) at 30µg/kg/min whereas at 100 and 300µg/kg/min no significant effects on heart rate were observed. Diastolic blood pressure decreased from 104 ± 7 to 61 ± 4 mm Hg whereas enddiastolic left ventricular pressure and mean pulmonary artery pressure increased from 10.4 ± 0.7 to 12.5 ± 0.6 mm Hg and 14.3 ± 5.0 to 15.7 ± 1.8 mm Hg, respectively. Coronary blood flow markedly increased from 39.3 ± 3.3 to 80.1 ± 7.8 ml/min in the arteria circumflexa.

In the second series of experiments OPC-8212 (5, 10 and 30 mg/kg) was orally administered in hard gelatine capsules to conscious chronically instrumented dogs (n=4). OPC-8212 induced a dose dependent increase in LV-dp/dtmax whereas no significant changes in heart rate or diastolic blood pressure were observed. Maximal effects on LV-dp/dtmax were observed after 1-2 hours after oral administration and these effects lasted for more than 5 hours. OPC-8212 in doses of 5, 10 and 30 mg/kg p.o. increased LV-dp/dtmax by 24 ± 3 %, 28 ± 4 % and 40 ± 7 %, respectively.

These results demonstrate that OPC-8212 is a positive inotropic agent with minimal effects on arterial blood pressure and heart rate. OPC-8212 induced increases in LV-dp/dtmax after oral administration to conscious dogs however these effects were observed at rather high doses.

EFFECTS OF BAY K 8644 ON HEMODYNAMICS AND REGIONAL VASCULAR CONDUCTANCE IN RABBITS.

H. Boss, T. Dietze and J.C.A. van Meel, Dept. of Cardiovascular Research, Dr Karl Thomae GmbH, D-7950 Biberach/Riss, FRG.

The so-called calcium agonist Bay k 8644 (methyl-1,4-dihydro-2,6-dimethyl-3-nitro-4-(2-trifluoro-methylphenyl)-pyridine-5-carboxylate) exerts vasoconstricting and positive inotropic effects (Schramm et. al., 1983). The aim of this study was to investigate the hemodynamic effects of Bay k 8644 in anaesthetized rabbits.

In the first series of experiments, Bay k 8644 was intravenously administered to pentobarbitone-anaesthetized rabbits (male Chinchilla, 3-4 kg). Dose-dependent increases in LV-dp/dtmax and diastolic blood pressure were observed. Maximal effects were obtained at 300 μg/kg of Bay k 8644. This dose increased LV-dp/dtmax by 2955 $\pm$ 210 mm Hg/s (n=6) and diastolic blood pressure by 76 $\pm$ 8 mm Hg (n=6). Heart rate decreased by 53 $\pm$ 10 bpm (n=6).

In the second series of experiments, Bay k 8644 was infused at 0.5, 1, 2 and 4 μg/kg/min (5 minutes for each infusion) into pentobarbitone-anaesthetized open-chest rabbits. Regional vascular conductance was measured using radiolabeled microspheres. Pronounced vasoconstricting effects of Bay k 8644 were observed in heart (right ventricle, septum, atrium, LV papillary muscle), and brain (cerebral cortex, cerebellum, medulla oblongata) whereas adrenal gland, kidney cortex, stomach wall, spleen, liver and jejunum were less influenced.

The results demonstrate that Bay k 8644 exerts pronounced hypertensive as well as positive inotropic effects in rabbits. The increases in arterial blood pressure are provoked by decreases in regional vascular conductances in various organs. The vasoconstricting effects of Bay k 8644 are very pronounced in the heart and the brain.

Schramm et al., Nature 309, 535-537 (1983).

606P

CARDIAC FUNCTION IN INTACT CONSCIOUS RATS: EFFECTS OF POSITIVE INOTROPIC AGENTS AND CORONARY ARTERY LIGATION.

J.J. Debets, R.G. Schoemaker, J.F.M. Smits and H.A.J. Struyker-Boudier, Dept. of Pharmacology, University of Limburg, Maastricht, The Netherlands.

Traditionally, in vivo studies of positive inotropic drugs are performed in large animal models for heart failure. In the present study we investigated some of the haemodynamic characteristics of a possible model for heart failure in rats, i.e. coronary artery ligated rats. In parallel, we studied the effects of known positive inotropic agents on cardiac performance in normal rats.

Heart failure is characterized by a depression of the Frank-Starling curve, relating cardiac filling pressure to cardiac performance. A positive inotropic agent should result in a steeper curve. These assumptions were used to develop a technique for assessment of cardiac function in our animals. Under pentobarbital (55 mg/kg, i.p.) anaesthesia Wistar rats were implanted with an electromagnetic flow probe on the ascending aorta to measure cardiac output (CO). Catheters were implanted in the abdominal aorta and thoracic vena cava for measurement of mean arterial (MAP) and central venous pressure (CVP). Furthermore, catheters were placed in the abdominal vena cava for infusions. Following surgery rats were allowed 4-6 days for recovery. Coronary artery stenosis was induced by ligation of the left descending coronary artery. On the experimental day CO was measured after hemodynamic parameters had stabilized both at rest and during rapid i.v. infusion of 12 ml Ringer's solution in 1 min.

Before coronary artery stenosis this intervention increased CVP from 2.4±0.7 to 11.3±0.3 cm H_2O (mean ±SEM; n=4) and CO rose from 84±5 ml/min to a plateau level of 145±15 ml/min. One day after coronary artery stenosis fluid loading increased CVP from 1.8±1.9 to 19.1±0.8 cm H_2O, whereas CO was not affected (from 62±15 to 63±22 ml/min).

In the parallel part of the study the effect of two known positive inotropic agents amrinone (A) and dobutamine (D) were studied in normal, unstenosed rats. After equilibration the rats were infused with saline, A (10-100 ug/kg/min; n=4-8 per dose) or D (20-200 ug/kg/min) for 30 min. Then stimulated CO was determined by volume loading. During saline infusions CVP increased from 2.1±0.7 to 17.5±2.3 cm H_2O and CO rose from 91±6 ml/min to a plateau level of 137±5 ml/min. A did not affect CO at rest (101±4 ml/ min during saline; 108±6 ml/min during 100 ug/kg/min). CO during volume loading (CO_{vl}) increased dose-dependently (196±5 ml/min during 100 ug/kg/min). D increased resting CO (148±3 ml/min during 200 ug/kg/min) and CO_{vl} (207±4 ml/ min during 200 ug/kg/min) dose-dependently.

These results suggest that cardiac function can be studied in the intact conscious rat under normal and pathophysiological conditions. The data indicate that coronary artery ligation suppresses the cardiac function curve. Furthermore the data indicate the potential value of this animal model in the study of positive inotropic drugs.

THE DESIGN OF NOVEL ANGIOTENSIN CONVERTING ENZYME INHIBITORS WITH β-ADRENOCEPTOR BLOCKING ACTIVITY.

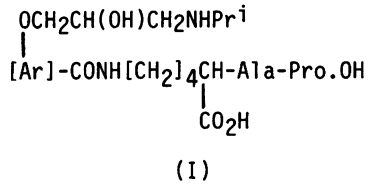
G. Allan, D. Bull, H.T. Jones, G.W. Hardy* and G. Mills, Departments of Pharmacology and Medicinal Chemistry, Wellcome Research Laboratories, Beckenham, Kent, UK.

Angiotensin converting enzyme (ACE) inhibitors, either alone or in combination with other agents such as beta-blockers or diuretics, have emerged as an effective treatment for many forms of hypertension (MacGregor et al, 1985). This study describes a novel series of ACE inhibitors which also have beta-blocking properties and are thus potentially useful antihypertensive agents.

Previous attempts to combine two complementary biological activities in one molecule have met with only limited success (Nicolaus, 1983). The design of compounds which exhibit in a single molecule both ACE inhibition and beta-adrenoceptor blocking properties took into account the fact that both ACE inhibitors and beta-blockers have compatible structural requirements for activity. We chose to synthesise a series of compounds, of general structure (I), which combine an ACE inhibitor of the enalapril-type with known beta-blockers.

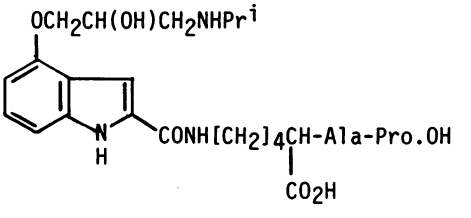
ACE inhibition and beta-adrenoceptor antagonism in vitro were determined as previously described (Allan et al, 1986). All of the compounds prepared were very potent ACE inhibitors [IC_{50}: 10 - 25nM, cf. enalaprilat 4.4 ± 0.8nM) but their potencies as beta-blockers were low (pK_b <6.0) except for analogues derived from pindolol which were found to be active (pK_b 6.2 - 7.2). Of this group, a compound designated BW A575C, ([N-(1-(S)-carboxy-5-[4-(3-isopropylamino-2-(R,S)-hydroxypropoxy)indole-2-carboxamido]pentyl)-(R,S)-alanyl-(S)-proline), was found to be a potent inhibitor of ACE whilst displaying appreciable beta-adrenoceptor blocking activity [IC_{50}: 10.7 ± 2.1nM; pK_b: 7.18 ± 0.05].

In conclusion, a study of compounds designed to exhibit both ACE inhibition and beta-adrenoceptor antagonism has led to the discovery of an active agent, BW A575C, which has been chosen for further investigation as a potential antihypertensive agent.



(I)

Ar = benzene, naphthalene or indole



BW A575C

Allan, G. et al, (1986). Proc. Brit. Pharmacol. Soc., (this meeting)
MacGregor, G.A. et al, (1985). J. Cardiovasc. Pharmacol., 7, Suppl. 582-587
Nicolaus, B.J.R., in "Decision Making in Drug Design", Ed. F. Gross, Raven Press, New York, 1983, pp173-187.

FLUORIDE-INDUCED CONTRACTION IN RAT AORTA.

G. Martin, M. Schachter, P.S. Sever. Department of Clinical Pharmacology, St. Mary's Hospital Medical School, Norfolk Place, London, W.2.

Fluoride is known to interact with guanine nucleotide binding proteins which activate (Ns) or inhibit (Ni) adenylate cyclase (Katada et al., 1984). Recent evidence indicates that in the rat hepatocyte (Blackmore et al., 1985) and in the neutrophil (Strnad and Wong, 1985), fluoride may also produce intra-cellular calcium mobilisation and calcium influx. In the case of the neutrophil this effect is not inhibited by pertussis toxin, suggesting that neither Ni nor Ns are involved. The effect of pertussis toxin on the hepatocyte is not known, but the existence of a novel guanine nucleotide binding protein has been suggested in studies using calcium-mobilising hormones (Uhing et al., 1986; see also Rodbell, 1985). We have examined the effect of fluoride on vascular smooth muscle contraction, a process which is critically dependent on cytosol calcium concentration.

Male Sprague-Dawley rats were killed by thoracic stunning and the thoracic aortae were rapidly dissected. Rings of aorta were suspended between stainless steel hooks in organ baths containing Krebs ringer buffer at 37°C, gassed with O_2 95% / CO_2 5%. The rings were maintained at a resting tension of 1 gm for 90 min. Subsequent responses were recorded using Grass FT03 isometric transducers connected to a Grass 79D polygraph. The following results are derived from 3-12 experiments.

Sodium fluoride (F) at concentrations of 3 mM or more, produced contraction in all segments tested (mean max. tension = 1.91 gm $\pm$ 0.17). Relaxation could be induced by repeated washing, following which response to F was undiminished. Tachyphylaxis was not observed in normal Kreb's buffer even after 3 applications of F. Cumulative concentration-response (CCR) curves showed an EC50 for F of 7.2 mM, with maximal response at 18mM. F-induced contractions were not inhibited by nimodipine (1 μM) or diltiazem (1 μM). In calcium-free Krebs buffer with 2 mM EGTA, the CCR curve was shifted to the right and indicated a non-competitive effect (EC50 = 9.3 mM, max. tension 0.83 gm $\pm$ 0.08). After stimulation with 18 mM F for 15 min, the preparation was washed 5 times, allowed to equilibrate for 15 min, and another CCR curve was obtained, with further diminution of contractile response (EC50 not determined, max. tension 0.26 gm $\pm$ 0.02). Pre-treatment of segments with sub-threshold concentrations of F (1 mM) did not influence subsequent responses to 80 mM KCl or 100 nM prostaglandin $F_2\alpha$. Pertussis toxin (1 μg/ml) did not attenuate F-induced contraction.

This study shows that F, 3-18 mM, produces reversible contraction of rat aortic smooth muscle. This response is partially dependent on extracellular calcium, but almost certainly also involves mobilisation of intracellular calcium stores. When calcium influx occurs it does not involve voltage-dependent calcium channels. By analogy with other systems, it is probable that a guanine nucleotide binding protein is involved in the action of F. However, as in the neutrophil, the response is not influenced by pertussis toxin. The mechanisms involved are currently being investigated.

Blackmore, P.F. et al. (1985). J. Biol. Chem., 260; 14477-14483.
Katada, T. et al. (1984). J. Biol. Chem., 259; 3568-3577.
Rodbell, M. (1985). Trends Biochem. Sci., 10; 461-464.
Strnad, C.F. & Wong, K. (1985). Biochem. Biophys. Res. Comm., 133; 161-167.
Uhing, R.J. et al. (1986). J. Biol. Chem., 261; 2140-2146.

A COMPARISON OF RECEPTOR-EFFECTOR COUPLING OF HISTAMINE AND ANALOGUES WITH THAT OF CARBACHOL IN THE GUINEA-PIG TAENIA CAECI.

Judith M. Hall, Deborah V. McCabe & I.K.M.Morton, Department of Pharmacology, King's College London, Strand, London WC2 2LS.

Histamine H_1 and muscarinic receptors may well share a common effector pathway in intestinal smooth muscle, but there are indications from electrophysiological, ion flux, phosphatidylinositol (PI) hydrolysis, and contraction experiments, that histamine is less effective than carbachol.

We have studied these agonists for similarities in their mechanisms for the translocation of calcium in the guinea-pig taenia caeci,and have investigated some possible reasons for the relative ineffectiveness of histamine at receptor level.

In experiments where the contribution of voltage-operated ion channels (VOCs) was abolished by using Ca^{2+} containing high K^+-solutions the contraction might then be considered to result from an increase in Ca^{2+} entry through receptor-operated channels (ROCs) and/or release of Ca^{2+} from intracellular stores (Hall & Morton, 1986). Under these conditions the maximum contractile response of histamine as compared to carbachol was reduced even further, also ^{86}Rb efflux experiments detected little ROC opening by histamine whereas carbachol was very effective. This suggests that release of Ca^{2+} from internal stores may be very important for H_1 receptors, and indeed caffeine (10mM), which can deplete Ca^{2+} from the sarcoplasmic reticulum,abolished the response to histamine whilst inhibiting that to carbachol by only half.

The response of a receptor system also depends (amongst other factors) on the intrinsic efficacy (ε) of the agonist and the receptor density (R_T). Therefore several histamine analogues of potentially differing ε (and that had previously been quantified for PI response in guinea-pig ileum (Donaldson & Hill, 1985)), were evaluated relative to histamine for contractile activity under various conditions, effect on ^{86}Rb efflux; and also K_D values and receptor reserve were estimated by the phenoxybenzamine method. The H_1 analogues were; N^{α}-methyl histamine, N^{α}, N^{α}-dimethyl histamine, 2-thiazolylethylamine, 2-pyridylethylamine and 2-methyl histamine.

Briefly, these experiments suggest that Ca^{2+} mobilization within the cell is by a combination of entry and release. Of the histamine analogues N^{α}-methyl histamine is at least as efficacious as histamine; moreover the system behaves as though the density of H_1 receptors is lower than that of muscarinic receptors.

J.M.H. is an M.R.C. Scholar. We thank S.K. & F. for their generous donation of histamine analogues.

Donaldson, J. & Hill, S.J. (1985). Br.J.Pharmac. 85, 499 -512

Hall, J.M. & Morton, I.K.M. (1986).Br.J.Pharmac. 87, 206P.

OPIOIDNEUROMODULATION OF CHOLINERGIC NERVE ACTIVITY IN HUMAN AND GUINEA-PIG COLON.

D E Burleigh and S J Trout. Department of Pharmacology & Therapeutics, The London Hospital Medical College, Turner Street, London E1 2AD.

This investigation seeks to compare the effects of morphine on acetylcholine release from the guinea-pig colonic smooth muscle-myenteric plexus preparation (SMMP; Trout 1986) and human taenia coli muscle strips (Burleigh & Trout 1986). Preparations were incubated in [^{3}H]-choline to radiolabel neuronal stores of acetylcholine and then washed out for 90 min. The preparations were stimulated twice and the superfusate sampled for tritium content. Radioactive release was expressed in terms of a fractional release ratio (FRR) of the two stimuli (Rand et al, 1982) so that control ratios could be compared to ratios where drugs were introduced between the stimuli. Morphine depressed evoked release of tritiated material from both preparations in a concentration dependent manner (table 1).

Table 1 The effect of morphine (M) on release of [^{3}H] material (median and inter-quartile FRR values) from human taenia and guinea-pig colonic SMMP.

	Guinea-pig colonic SMMP			Human taenia †	
	1 Hz	10 Hz		1 Hz	10 Hz
Control	**0.74** (0.89-0.63)	**0.89** (0.96-0.86)	Control	**0.84** (0.90-0.78)	**0.83** (1.27-0.78)
M 0.26 µM	**0.69** (0.86-0.63)	**0.71** (0.74-0.65)	M 0.13 µM	**0.82** (0.88-0.59)	**0.67** (0.76-0.53)
M 2.60 µM	**0.51** (0.58-0.49)	**0.71** (0.71-0.68)	M 1.30 µM	**0.60 *** (0.65-0.55)	**0.88** (0.96-0.86)
M 26.0 µM	**0.40 *** (0.48-0.36)	**0.66 *** (0.85-0.65)	M 13.0 µM	**0.50 *** (0.59-0.45)	**0.63** (0.79-0.63)

* $P < 0.05$ Mann Whitney U test † From Burleigh & Trout 1986 $n \geq 5$

Preliminary experiments with loperamide using the SMMP preparation have demonstrated a greater inhibition of [^{3}H] release than that obtained with the highest dose of morphine. Loperamide at 0.2, 2.0 and 20.0 µM gave fractional release ratio values of 0.49 (0.85-0.30), 0.23 (0.29-0.10) and 0.00 (0.00-0.00) respectively compared to 0.87 (0.90-0.80) with the solvent control. A possible explanation is that at the higher concentrations, loperamide has calcium blocking activity (Reynolds et al, 1984). In the present study [^{3}H] release has been shown to be calcium sensitive, also contractions to acetylcholine were abolished by loperamide (20 µM).

With the protocol adjusted to investigate naloxone and morphine simultaneously in the SMMP preparation, morphine (2.6 µM) caused a significant ($P < 0.05$) reduction in [^{3}H] release. Naloxone (0.27 µM) alone significantly ($P < 0.05$) enhanced the FRR yet in the presence of this concentration of naloxone, morphine (2.6 µM) did not cause a significant ($P > 0.05$) reduction in release.

The data presented here indicates that morphine has a depressant action on cholinergic nerve activity in both preparations tested. In addition, further evidence is presented suggesting a non-opioid effect of loperamide.

Burleigh D E and Trout S J (1986). Br J Pharmac 88 307-313.
Rand M J, Story D F and Wong-Dusting H (1982). Br J Pharmac 76 305 - 311
Reynolds I J, Gould R J and Snyder S H (1984). J Pharmac Exp Ther 231 628 - 632
Trout S J (1986). J Pharm Pharmac 38 310-312

THE EFFECTS OF ACETYL-STROPHANTIDINE ON BODY SURFACE LATE POTENTIALS IN INFARCTED DOGS.

C .D.J. de Langen*, J.H. Levine[1], E.N. Moore[1], J.F. Spear[1], Department of Pharmacology/Clinical Pharmacology, University of Groningen and [1]Department of Animal Biology, School of Veterinary Medicine, University of Pennsylvania, Philadelphia.

The effects of the cardiac glycoside acetyl-strophantidine on the signal averaged electrocardiogram were assessed in eight anaesthetized (30 mg/kg Na pentobarbital) mongrel dogs 8 to 10 weeks after left anterior descending coronary artery occlusion and reperfusion.

Averaged X, Y and Z complexes were vector summed and filtered at 50 Hz. After an initial recording, acetyl-strophantidine (0.5 mg plus 0.25 mg every 10 min) was administered untill toxicity developed in all dogs (frequent premature beats in 6 out of 8, accelerated idioventricular rhythm in 2 out of 8, additional intestinal spasms in 3 out of 8). The initial sinus rhythm returned after 13 $\pm$ 4 min and a second recording was made.

Sinus cycle length was unaffected by the drug (101 $\pm$ 3% of control). The PQ interval was prolonged from 102 $\pm$ 3.0 to 104 $\pm$ 2.8 ms ($p < 0.05$). The duration of the low amplitude (< 30 μV) terminal part of the averaged QRS-complex was prolonged to 127.3 $\pm$ 9.5% of control ($p < 0.05$). In contrast, the initial, high amplitude part of the QRS complex was not affected.

Apparently, the late activation in infarcted areas was further delayed by the cellular decoupling effect of the glycoside (Weingart, 1977). Conduction in the infarcted tissue, where abnormal electrophysiologic properties, related to a "mottled", heterogeneous anatomy are present (Spear *et al.* 1983a; Spear *et al.* 1983b) might be more susceptible to cardiac glycosides, causing ventricular arrhythmias.

Spear, J.F., Michelson, E.L. & Moore, E.N. (1983a) Circ. Res. 53: 176-185.

Spear, J.F., Michelson, E.L. & Moore, E.N. (1983b) J. Am. Coll. Cardiol. 1: 1099-1110.

Weingart, R. (1977) J. Physiol. 264: 341-365.

COMPARTMENTATION OF CYCLIC GMP IN THE RABBIT MYOCARDIUM.

M. Dickson, R.J. Marshall and M. Shahid, Department of Pharmacology, Organon Laboratories Limited, Newhouse. ML1 5SH

Many investigators have attempted to elucidate the role of cyclic nucleotides in myocardial function. Singh and Flitney (1981), using the frog heart, showed that cyclic AMP and cyclic GMP modulate cardiac contractility by producing opposite inotropic effects. In contrast more recent work (Rodger and Shahid, 1985) has shown that no such obvious relationship, between the nucleotides, exists in the mammalian heart. The aim of the present study was to further clarify the role of cyclic GMP, using agents that selectively modify cyclic nucleotide levels, in regulating cardiac force.

Papillary muscles, from male New Zealand white rabbit hearts, were suspended in modified Krebs solution containing (mmol l^{-1}): NaCl 118, KCl 4.7, $MgSO_4.7H_2O$ 1.2, KH_2PO_4 1.2, $CaCl_2.6H_2O$ 2.5, $NaHCO_3$ 25, glucose 11.7 and electrically stimulated frequency of 0.4Hz. All experiments were performed in the presence of propranolol ($1x10^{-7}M$) to eliminate β-adrenoceptor involvement. The exact procedure for measuring contractile responses and cyclic nucleotide levels has been described in detail elsewhere (Rodger and Shahid, 1985).

Table 1 Carb.=carbachol; Atrop.=atropine; SNP=sodium nitroprusside

Treatment	Tension (mg) -drug	Tension (mg) +drug	cAMP(pmol/mg)	cGMP(pmol/mg)
Control (Propranolol, $10^{-7}M$)	388±54	-	0.763±0.079	0.0250±0.0065
Carbachol (22μM)	413±78	506±79	0.631±0.037	0.0583±0.0130*
+Atrop. (1μM)	467±37	428±38	0.925±0.156	0.0220±0.0099
Forskolin (2.5μM)	617±67	2167±73	5.051±0.372	0.0362±0.0100
+carb. (22μM)	1478±173	819±112	5.493±0.429	0.0914±0.0160**
+carb.+Atrop. (1μM)	1418±138	1464±153	4.378±0.637	0.0457±0.0100
+SNP (1mM)	1350±275	1463±284	4.319±0.959	0.2308±0.0373**
+SNP+Atrop. (1μM)	1468±205	1554±223	4.863±0.806	0.1997±0.0376**
+SNP (1mM)+carb. (22μM)	2100±215	1400±193	5.693±0.930	0.3080±0.0504**
+SNP+carb.+Atrop (1μM)	2206±268	2122±271	3.413±0.472	0.2423±0.0454**

n=4-9; * $p<0.05$; ** $p<0.01$ (Student's t-test) compared to control

The results (Table 1) show that carbachol alone exerts no inotropic effect despite increasing cyclic GMP levels. However, carbachol does produce a negative inotropic effect when added after forskolin. The carbachol-induced partial reversal of the positive inotropic effect of forskolin and the associated increase in cyclic GMP levels are both blocked by atropine. In contrast, SNP, despite producing a ten-fold increase in cyclic GMP did not depress the cardiotonic action of forskolin. Addition of carbachol after SNP, in the presence of forskolin, still produced an atropine-sensitive negative inotropic effect. Other experiments showed that carbachol did not affect the positive inotropic action of agents acting through a cyclic AMP-independent mechanism.

It is concluded that carbachol produces a negative inotropic effect, in papillary muscles, only when cyclic AMP levels have been initially elevated and that the muscarinic-receptor mediated increase in cyclic GMP appears to be involved in this action.

Rodger, I.W. and Shahid, M. (1985) Br. J. Pharmac. 81, 151-159
Singh, J. and Flitney, F.W. (1981) Biochem. Pharmacol. 30, 1475-1481

THE ANTIARRHYTHMIC PROPERTIES OF NALOXONE AND MrZ 2593 (NALOXONE METHOBROMIDE) IN VIVO AND IN VITRO.

J.E. Mackenzie*, J.R. Parratt and Rebecca Sitsapesan, Department of Physiology and Pharmacology, University of Strathclyde, Glasgow and Pharmaceutical Division, Reckitt and Colman, Hull*.

It has been shown that naloxone is antiarrhythmic both in conscious and anaesthetised rats (Fagbemi et al., 1982). However, it is not known whether this action is mediated centrally or peripherally. Hence, the aim of this study was to compare the effects of naloxone on the arrhythmias resulting from coronary artery occlusion in anaesthetised rats and in rat isolated hearts and to determine whether MrZ 2593, which is a quarternary complex of naloxone and does not enter the central nervous system (CNS), is antiarrhythmic in anaesthetised rats.

Sprague-Dawley rats (250-350g) were anaesthetised with pentobarbitone and artificially ventilated. Drugs or saline were administered intravenously 15 min before coronary artery occlusion using the method of Clark et al.,(1980). Langendorff perfused rat hearts were made ischaemic by coronary artery occlusion 10 min after starting naloxone perfusion and at the corresponding time with control hearts. The severity of the arrhythmias was assessed in the 0-30 min post-occlusion period (Clark et al., 1980).

In anaesthetised rats, naloxone ($0.5 mgkg^{-1}$ followed by $0.25 \mu gkg^{-1}min^{-1}$ by infusion) significantly reduced the total number of ventricular ectopic beats (VEB's) from 1078±305 to 213±70 ($P<0.05$) and the duration of ventricular tachycardia (VT) from 65±26s to 5±1s ($P<0.05$). At $2 mgkg^{-1}$ + $1 \mu gkg^{-1}min^{-1}$, naloxone significantly reduced VEB's from 1307±346 to 463±144 ($P<0.05$) and the % incidence of ventricular fibrillation (VF) from 90% to 30% ($P<0.05$). In contrast naloxone (10µM) failed to reduce the arrhythmias occurring after coronary artery occlusion in rat isolated hearts.

MrZ 2593 in the anaesthetised rat model was antiarrhythmic at a dose of $1 mgkg^{-1}$ and significantly reduced the duration of VT from 118±43s in the controls to 11±5s ($P<0.05$).

These results could indicate that naloxone may have a peripheral site/sites of action since MrZ 2593, which does not enter the central nervous system, like naloxone possesses antiarrhythmic properties.

The lack of effect of naloxone in isolated heart preparations suggests that a postsynaptic action is perhaps unlikely.

This study was supported by SERC and by Reckitt and Colman Ltd., Hull*.

Clark, C., Foreman, M.I., Kane, K.A., McDonald, F.M. & Parratt, J.R. (1980) J. Pharmac. Methods, 3, 357-368.
Fagbemi, O., Lepran, I., Parratt, J.R. & Skekeres, L. (1982). Br. J. Pharmac., 76, 504-506.