VERAPAMIL AND CALCIUM CHELATING AGENTS INCREASE THE NEUROMUSCULAR BLOCKADE PRODUCED BY PANCURONIUM IN THE CHICK SKELETAL MUSCLE

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It has been reported that verapamil,an organic calcium antagonist or entry blocker, intensifies pancuronium-induced neuromuscular blockade in both vitro and vivo (Bikhazi,Leung & Foldes,1982;Durant,Nguyen & Katz,1984),possibly by inhibiting Ca^{2+} transport through cell membranes (Van Der Kloot & Kita,1975).The effect of calcium chelating agents,e.g EDTA,EGTA,inorganic calcium antagonists,on neuromuscular transmission and blockade has not been studied and the aim of the present investigation was to study and compare the neuromuscular effects of some calcium chelating agents ,verapamil,and pancuronium in chick isolated skeletal muscle.

The preparation,chick biventer cervicis nerve-muscle (Ginsborg & Warriner,1960), was set up in an organ bath containing Krebs-Henseleit solution (20 ml) maintained at 38± 2°C and bubbled with 5% carbon dioxide in oxygen.The mechanical responses produced by repetitive motor nerve stimulation,at 0.18 Hz with 5-10 V maximal voltage and 0.2 ms pulse duration,and drug application were recorded isometrically by means of a force-displacement transducer (Dl 50 g) and a George Washington pen recorder (model 400 MD 2C,Bioscience,U.K.).

Pancuronium (15-1500 nM),verapamil (1-100 µM),EDTA (0.07-7.0 mM),EGTA (0.1-10 mM) and lanthanum chloride (0.14-14 µM) reduced the indirectly-elicited twitch contractions in a dose-dependent manner.Verapamil (20 µM) and EDTA (0.7 mM) both significantly increased the neuromuscular blockade produced by pancuronium in the chick muscle.The mean (±s.e.m.) IC_{50} values (concentration to produce 50% inhibition of twitch tension) of pancuronium-induced blockade alone and in combination with either verapamil or EDTA were 216± 12 nM, 68± 4 nM, 98± 6 nM respectively(n=6), giving a dose-ratio (control/test) of 3.2:1.0 and 2.2:1.0 respectively. Similar results were obtained with EGTA (1 mM) and lanthanum chloride (1.4 µM). Increasing the external calcium concentration,from normal 2.5 mM ,to 5.0 mM only antagonized the inhibitory effect of the calcium chelating agent,leaving the effect of verapamil unaltered.Recovery from verapamil ,alone,or in conjugation with pancuronium was difficult,compared to that following the calcium chelating agent and pancuronium (5-10 min).

In conclusion,both organic (verapamil) and inorganic (EDTA,EGTA) calcium antagonists are capable of intensifying the pancuronium-induced neuromuscular blockade in the chick skeletal muscle.Verapamil intensification of pancuronium blockade was more pronounced than the chelating agent,and the results further suggest that the calcium antagonists,entry blocker and chelator,may act by different mechanism at the chick skeletal muscle(Van Der Kloot & Kita,1975;Bikhazi,Thomas & Foldes,1979).

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METHAEMOGLOBINAEMIA PRODUCED BY p-AMINOPROPIOPHENONE AND p-AMINOOCTANOYLPHENONE

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The homologous series of p-aminophenones produce methaemoglobinaemia. In the case of one of these compounds, p-aminopropiophenone, the methaemoglobinaemia has been used in the treatment of cyanide poisoning. (Jandorf and Bodansky, 1946). Molar equivalent doses of p-aminovalerylphenone, p-aminohexanoylphenone and p-aminoheptanoylphenone which differ from p-aminopropiophenone by having longer aliphatic side chains produce lower peak methaemoglobin levels (Bright and Marrs, 1983) when given orally to beagle bitches. The duration of methaemoglobin does not greatly differ for the four compounds at equivalent doses but when the methaemoglobin profile was compared after intravenous dosing of bitches with p-aminopropiophenone and p-aminohexanoylphenone at equimolar doses peak levels of methaemoglobin occur about 10 min later with the latter than the former.

We have compared p-aminopropiophenone and p-aminooctanoylphenone at doses producing similar peak methaemoglobin levels by the oral and intravenous routes, in beagle bitches. p-aminopropiophenone and p-aminooctanoylphenone were administered orally at doses of 0.5 mg kg^{-1} and 22.5 mg kg^{-1} respectively to 3 beagle bitches. Methaemoglobin levels were estimated at 10 min intervals for 2½ h and at 3 h and hourly thereafter for up to 8 h, on an IL 282 CO-oximeter (Instrumentation Laboratories): p-aminopropiophenone and p-aminooctanoylphenone were also each administered intravenously to 3 beagle bitches at doses of 0.5 mg kg^{-1} and 13.5 mg kg^{-1} in 2 ml PEG 300 and methaemoglobin levels were measured for up to 11 h.

After oral administration of p-aminopropiophenone peak methaemoglobin levels occurred at 60 to 90 min. The mean peak level was 14.0 ± 1.2%. After oral administration of p-aminooctanoylphenone peak levels occurred at 300 min and the mean peak level was 10.3 ± 2.6%. The duration of the methaemoglobinaemia above ½ peak levels was 147 ± 16 min in the case of the first compound and greater than 360 min in the case of the second. After intravenous p-aminopropiophenone peak levels occurred at 60-70 min (20.1 ± 0.6%) whilst after p-aminooctanoylphenone, by the same route of administration, they occurred at 3-6 h and the mean peak level was 16.1 ± 1.2%. The duration of methaemoglobin aemia above ½ peak levels was 167 ± 19 min in the case of p-aminopropiophenone and 501 ± 116 min in the case of p-aminooctanoylphenone. After intravenous administration of p-aminooctanoylphenone methaemoglobin levels were still elevated at 11 h. Since the two aminophenones differed in the duration of their methaemoglobinaemia both after intravenous and oral administration the difference is not due to differential absorption rates from the gut and is more likely to lie in metabolism.

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ACUTE AGONIST REGULATION OF α_2-ADRENOCEPTORS IN VITRO IN THE RABBIT

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Exposure to high levels of a hormone or agonist often leads to diminished physiological responsiveness or desensitization. This has been widely studied with β adrenoceptors (Harden et al, 1983). However, the effects of agonist exposure on receptors have not been studied as extensively.

We have examined changes in α_2-adrenoceptor mediated responses and number following short term agonist infusions using the α_2-adrenoceptor on rabbit platelets. Experiments were performed on New Zealand white rabbits (2-3 kg). Acute infusions of the α_2-adrenoceptor selective agonist, α methyl noradrenaline (αMN)(2.5 μmoles/kg/h) were given for 2.5, 5 or 10 mins through a catheter in an ear vein to groups of six rabbits. The α_1-adrenoceptor agonist, phenylephrine (Phen) was infused into other groups of rabbits as a control. Blood samples were removed before and at the end of each infusion (10 ml blood anticoagulated with sodium citrate). The ability of adrenaline (ADR) (10^{-4}-10^{-10}M) to enhance ADP (10^{-6}M) induced aggregation was studied using the turbidometric method of Born & Cross (1963). All aggregation experiments were performed in the presence of propranolol (10^{-6}M) to inhibit any β adrenoceptor effects. To examine receptor number, radioligand binding assays were performed on whole platelets using [^{3}H] yohimbine (1.25-25 nM), non-specific binding was determined by 10^{-5}M phentolamine. The maximum number of binding sites and dissociation constant were calculated by Scatchard analysis (Bmax: fmol/10^8 platelets K_D : nM). There were no significant changes in receptor density (Bmax = 21±2, K_D = 7±3 pre; Bmax = 20±2, K_D = 7±2 after infusion) with ten minute αMN infusions (n=6). In contrast a time dependent reduction in responses to ADR as measured by platelet aggregation was observed as summarised in the table. ADP responses were not significantly changed.

Aggregation responses to adrenaline (Δ OD/min).

	2.5 min αMN		5 min αMN		10 min αMN		10 min Phen.	
	Pre	Post	Pre	Post	Pre	Post	Pre	Post
10^{-5}M	38±12	24±9	53±20	29±9*	48±13	31±11*	47±19	42±19
10^{-8}M	7± 7	5±7	18±16	8±4*	23±16	16±13*	22±17	28±18
Emax	44±1	52±6	58± 2	34±3*	45± 3	26± 4*	43± 4	37± 2

* $p < 0.001$ when compared to before infusion.

The maximum response was reduced with 5 and 10 minute αMN infusions and dose response curves shifted to the right. There was no change observed in either [^{3}H] yohimbine binding or platelet aggregation with 10 minute Phen infusions.

In a separate series of experiments, another agonist with α_2-adrenoceptor activity, ADR (0.05 μmoles/kg/hr) was infused for 10 days into the femoral vein via osmotic mini-pumps. These relatively long term infusions resulted in a decrease in [^{3}H] yohimbine binding to platelets (Bmax = 12±2, K_D = 5±2 after ADR compared to Bmax = 28±5, K_D = 8±4 in controls) and in aggregation. (E_{max} 36±2 ADR; E_{max} 47±3 controls). Thus chronic exposure to agonists resulted in a decrease in α-adrenoceptor number and response. Our results from the acute studies would be consistent with rapid agonist desensitization of the platelet α_2-adrenoceptor without change in number. A similar time scale for β-adrenoceptor desensitization has been reported and it has been suggested that this is due to agonist induced reversible receptor endocytosis (Staehelin & Simons, 1982).

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COMPARISON OF THE MYDRIATIC ACTIONS OF α_1- AND α_2-ADRENOCEPTOR AGONISTS IN THE ANAESTHETISED RAT

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The dual autonomic innervation to the iris exerts opposing actions: the sympathetic system dilates the pupil whereas the parasympathetic system constricts it. In the rat there is evidence that the parasympathetic tone to the iris is inhibited centrally by α_2-agonists, resulting in a passive mydriasis (Berridge et al, 1983; Hey et al, 1985). In order to distinguish between the α-adrenoceptor mechanisms thought to be involved in the regulation of pupil diameter, the present study has compared the mydriatic actions of the α_1-agonist cirazoline (Cavero et al, 1982) and the α_2-agonist clonidine after topical application onto the conjunctiva of rats.

Male Sprague-Dawley rats (300-350g) were anaesthetized with sodium pentobarbitone and a lateral tail vein was cannulated for drug administration. Pupil diameter was measured as previously described (Berridge et al, 1983). Rats were placed on their left side and solutions (25 µl) of clonidine (1% w/v) or cirazoline (0.1 or 0.3% w/v), in the pH range 5.5-6, were dropped carefully onto the conjunctiva of the right eye. Pupil readings were taken bilaterally at five minute intervals after drug application.

Topically-applied cirazoline (0.1 and 0.3%) induced a pronounced mydriatic response in the ipsilateral pupil, but had no effect on the contralateral pupil. The effect was fairly slow in onset (5-10 min), with the pupil diameter reaching a maximum value of 3.8±0.3 mm (n=5) between 30 and 40 min after 0.1% cirazoline. The more concentrated cirazoline solution (0.3%) induced a similar magnitude of response, but with a more rapid onset of action (<30 min). In each group the contralateral pupil was consistently less than 0.4 mm. The mydriatic action of cirazoline (0.1%) was antagonized in a dose-related manner following 30 min pretreatment with the α_1-antagonist prazosin (1 and 3 mg/kg, i.v.). Pretreatment with phenoxybenzamine (2 mg/kg, i.v.) was similarly effective, producing complete inhibition of the mydriatic response. In contrast, the highly selective α_2-antagonist idazoxan (0.3 mg/kg, i.v.) had no effect on the mydriatic action of cirazoline.

Clonidine (1%) induced a bilateral mydriatic response. The onset of the response in both eyes was fairly rapid (<5 min) and reached a maximum value between 15 and 30 min: ipsilateral pupil = 4.2±0.1 mm; contralateral pupil = 3.8±0.08 mm at 30 min. The mydriatic effect was reversed in both eyes by idazoxan (0.3 mg/kg, i.v.), but was unaffected by 30 min pretreatment with phenoxybenzamine (2 mg/kg, i.v.).

These results indicate that the α-adrenoceptors in the rat iris are of the $\alpha_1$subtype. The most likely location of these receptors is on the dilator muscle (Narita and Watanabe, 1982). The bilateral mydriatic action of clonidine is most likely to result from a central action following systemic absorption of the drug from the eye. This agrees with our previous finding that α_2-agonists induce mydriasis through a central α_2-mechanism, probably by suppressing parasympathetic tone to the iris sphincter (Berridge et al, 1983).

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EFFECTS OF OPTAZAPINE, MIANSERIN AND IDAZOXAN ON PRESSOR RESPONSES TO α-AGONISTS, TYRAMINE AND 5-HT IN PITHED RATS

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Mianserin is known to block α2- and α1-adrenoceptors, neuronal uptake of noradrenaline (NA) and 5-HT receptors (Cavero et al., 1979, 1981). Aptazapine (CGS 7525A) is a more potent α2-adrenoceptor antagonist and a weaker NA uptake inhibitor than mianserin (Liebman et al., 1983; Doxey et al., 1985). Aptazapine and mianserin possess similar affinity for 5-HT 2 binding sites labelled with 3H-spiroperidol (Liebman et al., 1983). We have compared the effects of aptazapine, mianserin and the selective α2-antagonist idazoxan on the pressor responses to α1- and α2-adrenoceptor agonists, tyramine and 5-HT in pithed rats.

Male Sprague-Dawley rats (275-350 g) were pithed and prepared for blood pressure measurement and i.v. drug administration as described previously (Doxey et al., 1983). Rats were pretreated with propranolol (1 mg/kg). Pressor responses to NA (0.5 µg/kg), tyramine (TYR: 100 µg/kg), cirazoline (CIR: 1 µg/kg) and UK-14,304 (UK: 5 µg/kg) were obtained before and 10 min after saline (1 ml/kg), aptazapine, mianserin and idazoxan (all 1 mg/kg) in separate groups (n=4-7) of pithed rats. Changes in diastolic blood pressure (DBP) to 5-HT (40 µg/kg) were obtained in separate groups of rats (n=5) pretreated 10 min before with either saline, aptazapine (0.1 - 1 mg/kg), mianserin (0.1 mg/kg) or idazoxan (1 mg/kg).

Control increases in DBP to NA, TYR, CIR and UK were 58±2 (n=23), 35±2 (n=20), 69±2 (n=23) and 51±2 (n=22) mmHg, respectively (pooled data). The responses to the four agonists were unaffected by saline. Idazoxan significantly inhibited the responses to NA (-29±6%), TYR (-26±5%), CIR (-13±5%) and UK (-58±4%). Mianserin significantly reduced the effects of TYR (-35±6%), CIR (-50±5%) and UK (-13±1%), but did not significantly alter NA (-6±4%). Similarly, aptazapine did not alter NA responses (-12±9%) whereas those to TYR (-22±5%), CIR (-48±2%) and UK (-48±3%) were significantly reduced. The findings that aptazapine and mianserin inhibited the responses to TYR, but not NA, is probably indicative of their NA uptake blocking properties. In saline pretreated rats, 5-HT evoked an initial transient pressor response of 44±3 mmHg followed by a more prolonged fall in DBP (-11±2 mmHg). Idazoxan did not alter the blood pressure effects to 5-HT. Mianserin markedly attenuated (-88%) the 5-HT induced rise in DBP and slightly potentiated its hypotensive effect. Aptazapine reduced the 5-HT pressor response; rises in DBP of 26±3 and 9±2 mmHg to 5-HT being noted after 0.1 and 1 mg/kg aptazapine. Unlike idazoxan and mianserin, aptazapine (1 mg/kg) significantly reduced (-51%) the peak fall in DBP induced by 5-HT. Aptazapine also reduced the duration of the 5-HT depressor response from 6.6±0.6 to 1.0±0.3 min.

In summary, only idazoxan appeared to be a selective antagonist of postjunctional α2-adrenoceptors, aptazapine was of similar potency at post α1- and α2-receptors whereas mianserin was more effective at α1-adrenoceptors. Aptazapine and mianserin produced effects consistent with blockade of NA uptake. Although these two compounds antagonised 5-HT, their profiles against this agonist were different. Further investigation into the effects of aptazapine on 5-HT is warranted.

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MUSCARINIC RECEPTOR SUBTYPES IN THE ILEUM, ATRIA AND TAENIA CAECUM OF THE GUINEA-PIG

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Muscarinic receptors (mAChRs) present on the ileum and atria have been classified into two subtypes on the basis of differential affinities of 4 diphenyl acetoxy N methyl piperidine methiodide (DAMP) and are denoted as m1 and m2 respectively (Barlow et al, 1976). In addition, mAChRs predominantly present on the sympathetic ganglia and smooth muscle have been classified according to the differential affinities of pirenzepine and are denoted as M1 and M2 respectively (Hammer & Giachetti, 1982).

The taenia is highly responsive, in comparison to the ileum and atria, to McN-A-343, a muscarinic agonist. McN-A-343 has been proposed as a selective M1 agonist, since it selectively stimulates mAChRs present on sympathetic ganglia (Hammer & Giachetti, 1982). The aim of the present study was to assess the mAChR subtype present on the taenia since it may represent a peripheral tissue in which M1 receptors predominate.

Guinea-pig ilea, atria and taenia were placed in Krebs bicarbonate buffer at 30°C. Antagonist affinities were determined according to the method of Arunlakshana and Schild (1959). Carbachol produced a response in all three tissues (ileum EC_{50} = 5.5 x 10^{-7} M; atria EC_{50} = 6.0 x 10^{-7} M and taenia EC_{50} = 5.1 x 10^{-7} M). McN-A-343 produced a response in the taenia only (EC_{50} = 7.3 x 10^{-6} M). The antagonist affinities using carbachol as the agonist are shown in Table 1.

Antagonist	Ileum	Atria	Taenia Caeci
Atropine	9.10	8.87	8.69
Pirenzepine	6.77	6.60	6.34
DAMP	9.04	7.90	8.73
Gallamine	4.84*	5.84*	4.12*

Table 1 Antagonist affinities at ileal, atrial and taenial receptors. Values are mean pA_2 values, n = 3 - 6 preparations at each concentration.
* indicates Arunlakshana-Schild slope significantly less than unity.

When McN-A-343 was used as the agonist, the affinity of pirenzepine was 6.86. The results indicate that the taenia has a similar profile to the ileum, since the differences are less than 0.5 pA_2 units, a criteria suggested by Furchgott (1972) as evidence for receptor heterogeneity. This indicates that the subtype may be classified as m1 according to the scheme of Barlow et al (1976). Secondly, the values indicate that the receptor may be classified as M2 according to the scheme of Hammer & Giachetti (1982) since the affinities obtained using pirenzepine are much lower than those reported for the receptors present on the sympathetic ganglion (8.4) by Brown et al (1980). Therefore, the taenia caeci appears not to be a tissue suitable for the study of M1 receptor function, even although the tissue is responsive to McN-A-343. The reason for the apparent selectivity of this agonist is probably a reflection of different receptor reserves in these tissues.

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COMPARISON OF α_2-ADRENOCEPTOR-MEDIATED PRESSOR RESPONSES IN PITHED RATS AND RABBITS

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It has been found *in vitro* that the order of potency of α-adrenoceptor antagonists differs when tested against α_2-adrenoceptor agonists in tissues from rat and rabbit (Alabaster & Peters, 1984; Waterfall *et al*, 1985). This might suggest sub-types of the α_2-adrenoceptor as has also been indicated from ligand-binding studies (Nahorski *et al*, 1985).

We have now tested this *in vivo* in the pithed rat and rabbit using the pressor response to noradrenaline (NA). Animals were pithed under halothane anaesthesia (Gillespie *et al*, 1970; McGrath & Mackenzie, 1977) and were artificially ventilated with oxygen and given propranolol (1 mg/kg, i.v. at 20 min intervals). Dose/diastolic carotid arterial pressor response curves to NA (0.01 - 10 µg/kg) were constructed and the α_2-mediated response was isolated by blocking the α_1-mediated response with a large dose of prazosin (1mg/kg). Two antagonists, rauwolscine and Wy 26703, were tested in each species over a similar concentration range (0.1-10mg/kg). All drugs were administered by bolus injection into the right jugular vein.

The rat was 10 times more sensitive to NA than was the rabbit. Prazosin, given on its own, produced a dose-related rightward shift in the NA dose/response curve in each species. In the rat these shifts were parallel and reached a rightward limit at prazosin 1mg/kg. In the rabbit the shift resulted in a shallower gradient for the NA dose/response curve and, again, reached a rightward limit at 1mg/kg. In each species, rauwolscine or Wy 26703 given on their own, produced small rightward shifts, with steepening gradient, as previously described in the rabbit for rauwolscine and another α_2 antagonist, imiloxan (McGrath & McKean, 1982). Given after prazosin (1mg/kg), each antagonist produced a further rightward shift, greater than would be found in the absence of prazosin. Again these shifts had a rightward limit, presumably when they reached the position to which prazosin had shifted the α_1 dose/response curve or at which non-α-mediated effects of NA appear (the NA doses at this point are 3 to 1000 µg/kg). In the rat rauwolscine and Wy 26703 were equipotent. In the rabbit rauwolscine was more potent than Wy 26703. Rauwolscine was more potent in the rabbit than in the rat. Wy 26703 was more potent in the rat than in the rabbit.

The results confirm the hypothesis that α_2-adrenoceptors may be different in rat and rabbit and demonstrate that this applies to those involved in pressor responses to a catecholamine *in vivo*. The difference is characterised by an increase in the potency of rauwolscine from rat to rabbit as well as a decrease for Wy 26703.

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

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EFFECTS OF ALLOXAN DIABETES ON α-ADRENOCEPTOR SENSITIVITY IN RAT VAS DEFERENS

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The effects of diabetes on post-junctional adrenoceptor sensitivity in the rat vas deferens have yielded results which are varied and conflicting (Foy & Lucas 1976, Tomlinson & Yusof 1983).

By use of epididymal and prostatic portions of rat vas deferens stimulated by exogenous agonists or by electrical field stimulation, this study aimed to re-examine the effects of diabetes on post-junctional α_1 adrenoceptor sensitivity and to assess its effects on the pre-junctional α_2 autoregulatory receptor. Diabetes which had been induced 6-12 weeks prior to removal of the vasa was produced by i.p. administration of 2 doses of alloxan, the first of $100mgkg^{-1}$ and the second $150mgkg^{-1}$ five days later. This resulted in mean blood glucose levels of 67.5 +/- 9.6mM as compared with 13.6 +/- 0.9mM in untreated age-matched controls. Tissues were suspended in Krebs-Henseleit solution at 39°C under a resting tension of 0.5g. Responses to the exogenous agonists noradrenaline(NA) and methoxamine(MOA), were recorded isotonically and post-junctional sensitivity was assessed by comparison of the ED50 values for the agonists in control and diabetic rats. Maximal responses to electrical field stimulation (single pulse, 1.0mS,300mA) were recorded isometrically and pre-junctional receptor sensitivity was assessed as ID50 values for xylazine-induced inhibition. Parallel shifts to the left in the concentration-effect curves were noted for NA and MOA in both portions of tissue from diabetic rats, suggesting increased post-junctional sensitivity. The ED50 values are shown in the table. Only the ED50 for MOA in prostatic tissues showed a significant decrease. Xylazine caused a dose-related reduction in response to stimulation in epididymal tissues and the ID50 values were significantly reduced in diabetic rats, suggesting pre-junctional hypersensitivity. Absolute tensions developed in response to stimulation did not differ between control and diabetic groups (1.44 +/- 0.15g and 1.41 +/- 0.09g respectively). Xylazine-induced reductions in response were also seen in prostatic tissues. The ID50 for diabetic rats was reduced but was not significantly different from controls. In prostatic tissues, tensions recorded in the absence of xylazine exhibited an enhanced response to stimulation, 1.11 +/- 0.17g and 1.88 +/- 0.20g for control and diabetic animals respectively.

We conclude that diabetes does result in changed adrenoceptor responses and that these changes are unevenly distrbuted along the length of the vas.

Tissue Portion	Agonist	Animal Group	n	Agonist Mean ED50 / ID50*	s.e.m.	
Epididymal	Noradrenaline	Diabetic	8	4.9 x 10-6M	1.1 x 10-6M	n.s.
		Control	7	5.8 x 10-6M	0.4 x 10-6M	
	Methoxamine	Diabetic	8	6.9 x 10-6M	0.8 x 10-6M	n.s.
		Control	8	9.0 x 10-6M	1.3 x 10-6M	
Prostatic	Noradrenaline	Diabetic	8	1.8 x 10-5M	0.3 x 10-5M	n.s.
		Control	8	2.0 x 10-5M	0.2 x 10-5M	
	Methoxamine	Diabetic	6	1.2 x 10-5M	0.2 x 10-5M	P<0.05
		Control	6	2.0 x 10-5M	0.3 x 10-5M	
Epididymal	Xylazine	Diabetic	6	2.1 x 10-8M*	1.0 x 10-9M	P<0.05
		Control	8	3.9 x 10-8M*	6.0 x 10-8M	
Prostatic		Diabetic	7	9.4 x 10-8M*	2.5 x 10-8M	n.s.
		Control	6	14.8 x 10-8M*	3.7 x 10-8M	

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PHARMACOLOGICAL PROFILE OF APTAZAPINE; A COMPARISON WITH MIANSERIN AND IDAZOXAN

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Aptazapine (CGS 7525A) is a benzodiazepine derivative which is structurally similar to mianserin (Liebman et al., 1983). In vivo aptazapine was a more potent central α2-adrenoceptor antagonist than mianserin although at α1-binding sites (labelled with 3H-prazosin) both compounds possessed similar affinity. In addition, aptazapine was less effective than mianserin as an inhibitor of noradrenaline reuptake (Liebman et al., 1983). We have compared the effects of aptazapine and mianserin with those of idazoxan at peripheral and central α1- and α2-adrenoceptors and neuronal reuptake of noradrenaline (NA).

All experiments were performed using Sprague-Dawley male rats (275-375 g). Affinities (Ki, nM) of the compounds at α1- and α2- sites were determined by displacing the saturable, specific binding of 3H-prazosin and 3H-idazoxan from rat cerebral cortical membranes (Doxey et al., 1984). The potency of the agents as inhibitors of NA reuptake was expressed as the concentration preventing 50% (Ki, nM) reuptake of 3H-NA into hypothalamic synaptosomes (Walter et al., 1984). In vivo antagonist potencies at peripheral and central α2-adrenoceptors are given as AD50 values (μ moles/kg; cumulative i.v. dose producing 50% reversal) against maximal agonist responses to clonidine (100 μg/kg, i.v.) on the stimulation-evoked contractions of the vas deferens in pithed rats (Doxey et al., 1983) and guanoxabenz (300 μg/kg, i.v.) on pupil diameter in pentobarbitone anaesthetised rats (Berridge et al., 1983).

Table 1. Antagonist potencies and affinities at α-adrenoceptors and at NA uptake sites (AD50 = μ moles/kg and Ki = nM)

TEST SYSTEM				PHARMACOLOGICAL ACTIVITY		
				Aptazepine	Mianserin	Idazoxan
Peripheral	2	AD50	vas def	0.7	>15	0.1
Central	2	AD50	mydriasis	0.7	10	0.1
Central	2	Ki	binding	8.8	82	3.1
Central	1	Ki	binding	68.0	103	91.0
NA uptake		Ki		3545.0	366	>20000

The data (Table 1) show that aptazapine is a more potent antagonist of peripheral and central α2-adrenoceptors than mianserin although it was weaker than idazoxan. An AD50 for mianserin could not be obtained in the vas deferens probably because of its uptake blocking properties which tend to reduce the stimulation-evoked contractions. From the radioligand studies, all three compounds had relatively similar affinities for α1-binding sites. Idazoxan was the most selective compound for α2-sites (α1/α2 ratio = 29), followed by aptazapine (α1/α2 ratio = 8). Mianserin was non-selective with an α1/α2 ratio of almost unity (Table 1). Aptazapine was about 10 times less potent than mianserin as an inhibitor of NA uptake. Idazoxan was the least active in this respect with a Ki of >20 μM. Therefore, in conclusion, aptazapine is a more potent and more selective α2-antagonist with less NA uptake blocking properties than mianserin. Idazoxan was the most potent and selective α2-adrenoceptor antagonist of the compounds tested and was devoid of detectable NA uptake blockade.

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THE EFFECTS OF ANGIOTENSIN CONVERTING ENZYME INHIBITORS ON THE ACTION OF DIURETICS IN THE RAT

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There have been several reports that, in the clinic, co-administration of the angiotensin converting enzyme inhibitors (ACEI) captopril and enalapril reduce the hypokalaemia normally associated with diuretic therapy (Griffing et al, 1983; Holland et al, 1983; Johnston et al, 1979; Kelly et al, 1983). However, whilst it has been reported that captopril antagonised the renal response to frusemide in sodium-restricted rats (Chiu et al, 1984), there are no reports of the interaction between ACEI and diuretics in animals without sodium depletion. This report describes the effects of the ACEI captopril, enalapril and cilazapril (Ro 31-2848) on the diuretic response to hydroflumethiazide and frusemide in the saline loaded rat.

Groups of 8 male AHH/R hooded rats (85-170g) were used. The animals were treated orally with either hydroflumethiazide, frusemide or vehicle daily for 10 days. On days 1 and 10, 30 min before diuretic administration, ACEI or vehicle was given orally. On days 1 and 10 the diuretics were given in 0.45% saline, 25 $ml.kg^{-1}$, as vehicle and animals were placed in individual metabolism cages immediately after dosing. Five h urine collections were made. Urinary volume and cation content (sodium and potassium, flame emission spectrophotometry) were measured. Animals were returned to their normal cages at the end of the diuretic determinations.

Both hydroflumethiazide, 6 $mg.kg^{-1}$, and frusemide, 50 $mg.kg^{-1}$, caused a consistent increase in urinary volume and cation excretion. These effects were antagonised by the co-administration of all 3 ACEI with the antagonism being more marked on day 10 than day 1. There was no qualitative difference between the action of the ACEI. The minimum doses of each drug causing a significant inhibition of the diuretic responses are given in Table 1 which shows cilazapril to be the most potent drug examined.

Table 1 Inhibition of diuresis by ACEI

ACEI	Minimum dose ($mg.kg^{-1}$ p.o.) antagonising diuretic response			
	Hydroflumethiazide		Frusemide	
	Day 1	Day 10	Day 1	Day 10
Captopril	45	15-45	150	15-45
Enalapril	15	5	5	1
Cilazapril	$\leqslant 5$	$\leqslant 5$	5	$\leqslant 1$

These results suggest that combination with ACEI should reduce the often undesirable cation loss normally associated with diuretic therapy of hypertension and support the clinical observation that co-administration of an ACEI decreases the need for potassium supplement during diuretic therapy. Furthermore it has been shown that the antihypertensive actions of these ACEI in SH rats are enhanced by co-administration of a thiazide diuretic (Natoff et al, 1985).

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INTERACTIONS BETWEEN ANGIOTENSIN II, THE SYMPATHETIC NERVOUS SYSTEM AND BLOOD PRESSURE OF THE PITHED RAT

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Interactions between the renin-angiotensin system and the sympathetic nervous system have been shown in vitro (Malik, Nasjletti, 1976) and in vivo in pithed normotensive rats (Hatton, Clough, 1982). Recent work has suggested an apparent rather than real interaction in the pithed rat, related to changes in baseline mean arterial pressure, MAP (De Jonge et al, 1983). In the present study we have compared the effects of captopril with sodium nitroprusside and haemorrhage in pithed rats. Changes in responses to nerve stimulation and i.v. noradrenaline have been related to changes in baseline MAP.

	Nerve Stimulation		i.v. Noradrenaline	
GROUP	Baseline MAP (mmHg)	Increase in MAP at 2Hz (mmHg)	Baseline MAP (mmHg)	Increase in MAP at 3ug/kg(mmHg)
Saline Control	46.09 ± 1.00 (n = 15)	102.5 ± 3.04	49.1 ± 0.91 (n = 10)	99.31 ± 3.81
C	31.72 ± 1.27[b]	59.94 ± 4.63[b]	34.66 ± 1.52[b]	57.76 ± 8.03[b]
C + AII	46.82 ± 2.77	93.14 ± 8.75	48.22 ± 3.22	81.7 ± 6.54
C + AVP	47.94 ± 2.72	104.56 ± 3.05	53.72 ± 2.6	89.26 ± 3.39
SNP	36.4 ± 3.51[a]	33.54 ± 5.01[b]	33.12 ± 2.17[b]	3.14 ± 0.94[b]
Haemorrhage	32.14 ± 1.96[b]	80.06 ± 3.84[b]		

n = 5 unless otherwise stated P<0.01 = a, P<0.001 = b
SNP = Sodium Nitroprusside 100µg/kg/min, C = Captopril 5mg/kg, AII = Angiotensin II 25ng/kg/mm, AVP = Arginine Vasopressin 1.5 -3.0 mU/kg/min.

The inhibitory effects of captopril, on responses to nerve stimulation and noradrenaline injection, were reversed by treatments restoring baseline MAP. Other treatments reducing baseline MAP to a similar level (sodium nitroprusside, haemorrhage) also reduced the responsiveness of the preparation, but by varying degrees. Hence there was no clear quantitative relationship between the level of MAP and reactivity per se. Whilst the effects of captopril in reducing responsiveness in the pithed rat may be partly due to changes in baseline MAP, there are difficulties of interpretation in the pithed rat when treatments change baseline MAP. Definitive studies would probably involve measurement of blood vessel tone, rather than global blood pressure values.

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EFFECTS OF INHIBITORS OF THE RENIN-ANGIOTENSIN SYSTEM ON CARRAGEENIN-INDUCED OEDEMA IN RATS

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Subplantar injection of carrageenin in the rat hind limb causes an acute inflammatory response which is potentiated by the mercapto-containing angiotensin-converting enzyme (ACE) inhibitors captopril and YS980. Since ACE is identical to kininase II, potentiation of the carrageenin response by ACE inhibitors may be caused by accummulation of bradykinin, rather than by inhibition of the renin-angiotensin system (Boura and Svolmanis, 1984; Suda et al, 1984). In the present study, we have compared the effect of the structurally diverse ACE inhibitors captopril, enalapril and teprotide, with that of the angiotensin II antagonist saralasin, in the carrageenin inflammation model.

Inflammation was induced in the hind paw of Sprague-Dawley rats by subplantar injection of carrageenin (10µg in 0.1ml), and measured as oedema formation by plethysmography at 30, 60 and 180 min. One group of rats was dosed s.c. with enalapril 1mg/kg, captopril 1mg/kg, or saline 0.2ml, 30 min prior to carrageenin. Another group was dosed s.c. with teprotide 1mg/kg, saralasin 5mg/kg, or saline 0.2ml, 15 min prior to carrageenin. In a separate experiment in conscious catheterised rats, blood pressure responses to angiotensin I (AI) 100-400 ng/kg i.v. were measured. Inhibition of AI responses by s.c. doses of teprotide 1mg/kg, saralasin 5 mg/kg, or saline 0.2 ml, were measured.

In the first group of rats, carrageenin injection after saline pretreatment caused an increase in paw volume of 0.25 ± 0.02ml (n = 10) within 30 min. This response was significantly potentiated by pretreatment with either captopril (0.54 ± 0.02ml, $P<0.001$, n = 10) or enalapril (0.51 ± 0.02ml, $P<0.001$, n = 10). In the second group of rats, carrageenin injection after saline pretreatment caused an increase in paw volume of 0.37 ± 0.05 ml (n = 10) within 30 min. This response was significantly potentiated by pretreatment with teprotide (0.52 ± 0.05ml, $P<0.05$, n = 10). In contrast, the response was slightly attenuated by pretreatment with saralasin (0.29 ± 0.02 ml, N.S., n = 10). This was not withstanding greater inhibition of AI pressor responses by saralasin 5 mg/kg s.c. (79.9 ± 3.1%, n = 5) than by teprotide 1mg/kg s.c. (42.9 ± 6.4 %, $P<0.001$, n = 5) in the catheterised rats at the same timepoint.

Hence 3 structurally diverse ACE inhibitors significantly potentiated the oedema response to subplantar injection of carrageenin. This was in marked contrast to the effects of the angiotensin II antagonist saralasin. It is concluded that potentiation of carrageenin-induced inflammation is probably a feature of ACE (kinase II)inhibition per se. There is no evidence to suggest a similar effect with inhibition of other components of the renin-angiotensin system.

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THE EFFECTS OF ENALAPRIL UPON BLOOD PRESSURE, PLASMA AND TISSUE ANGIOTENSIN CONVERTING ENZYME (ACE) IN THE RAT

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Studies involving acute administration of the ACE inhibitor enalapril have reported lack of correlation between anti-hypertensive response, in vivo inhibition of angiotensin I (AI) conversion to angiotensin II (AII) and in vitro inhibition of plasma ACE. This raises the possibility that efficacy could be most closely related to inhibition of ACE in vascular tissue rather than in plasma (Cohen & Kurz, 1982; Sweet et al, 1981). We have investigated the effects of acute administration of enalapril upon the relationship between blood pressure and changes occurring in plasma and tissue ACE activity in the sodium-deficient normotensive (NT) rat. Inhibition of ACE activity in plasma was assayed in vivo (radioenzymatic method adapted from Sander et al, 1980) and in vivo (inhibition of pressor responses to intravenous AI). Blood pressure and responsiveness to intravenous AI were assessed in the pentobarbitone anaesthetised animal.

Male Sprague Dawley rats were maintained on a sodium-deficient diet (sodium content <0.02%, B.P. Nutrition) for 3 weeks. Blood pressure, in vivo ACE activity and plasma and tissue ACE activity in vitro were measured 0.5-48 h after a single oral dose of enalapril (10 mg/kg) or vehicle (n=20/group 24 h post-dose; n=6-10 other time points). ACE activity was assayed in plasma and tissues (aorta, kidney, adrenal glands, lungs and mesenteric bed) before and after removal of enalapril from the samples (method adapted from Ulm & Vassil, 1982).

Enalapril produced significant reductions in blood pressure (0.5-24 h post-dose) with no effect on heart rate. Pressor responses to AI (300 ng/kg i.v.) were maximally inhibited (approximately 85%) at 1 h post-dose, thus preceding the maximum fall in blood pressure observed at 4 h post-dose. Inhibition of AI pressor response and blood pressure falls were sustained up to 24, but not 48 h post-dose.

Plasma ACE activity (units = nmol substrate hydrolysed/mg protein/unit time) was significantly inhibited 0.5 h (maximum inhibition) to 24 h post-dose, but not at 48 h. Removal of enalapril from plasma revealed a significant increase in total enzyme only at 24 h after dosing (control = 0.331 ± 0.019 units; enalapril treated 0.426 ± 0.018 units; $p<0.01$) - this increase in enzyme concentration was not sustained at 48 h. Significant inhibition of ACE activity was observed in lungs and mesenteric bed up to 24 h following enalapril administration with maximum inhibition being observed 0.5 h post-dose as in plasma samples. Inhibition was maintained in lung samples up to 48 h. Small but non-significant increases in tissue enzyme concentration were observed 24 h after dosing.

In the present study acute oral administration of enalapril produced falls in blood pressure, inhibition of AI pressor responses in vivo and inhibition of both plasma and tissue ACE activity in vitro, although differences exist in the relative rate of onset and duration of these events. Thus in the sodium-deficient NT rat the acute blood pressure lowering effects of enalapril may be attributed to inhibition of ACE activity in both plasma and tissues.

Enalapril was kindly supplied by Merck Sharp & Dohme.

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INFLUENCE OF ANGIOTENSIN II ON PRESSOR RESPONSES TO α-ADRENOCEPTOR AGONISTS IN THE PITHED RAT

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We have previously shown that a low dose of the angiotensin converting enzyme (ACE) inhibitor, teprotide (1mg/kg), selectively inhibited the late, nifedipine sensitive components of the pressor responses to α-adrenoceptor agonists (Grant & McGrath, 1984). Teprotide (1mg/kg) had no effect on the early, nifedipine-resistant component of these responses. We have further attempted to study the influence of AII on α-mediated pressor responses in three ways: (i) using a higher dose of teprotide, (ii) using the angiotensin II (AII)-receptor antagonist saralasin and (iii) infusing subpressor doses of AII.

Male Wistar rats (245-265g) were pithed and ventilated to produce normal arterial blood gas tensions. Boluses of drugs were administered in a fixed volume of 1ml/kg followed by a similar volume of saline. Drugs were infused at 1.5ml/hr.

Teprotide (10mg/kg) inhibited the pressor response to AI (0.5 ug/kg) by 87% indicating a marked inhibition of ACE. Teprotide (10 mg/kg) inhibited the peak pressor responses to noradrenaline (1ug/kg), to the α_1 agonist phenylephrine (3ug/kg) and to the α_2 agonist xylazine by 50%, 60% and 57% respectively and also eliminated the late, slow components of the responses. Teprotide (10mg/kg) also inhibited the maintained pressor response to noradrenaline infusion (1ug/kg/min) by 63%. Saralasin (4ug/kg/min) significantly inhibited the pressor response to AII (400ng/kg) by 72% indicating marked AII-receptor antagonism. Saralasin (4ug/kg/min) inhibited peak pressor responses to noradrenaline (1ug/kg), phenylephrine (3ug/kg) and xylazine (0.5mg/kg) by 35%, 33% and 52% respectively. Infusion of subpressor AII (50ng/kg/min) gradually, over 60min, potentiated pressor responses to noradrenaline (1ug/kg), to xylazine (0.1 mg/kg) and to the α_1 agonist indanidine (0.5mg/kg).

In conclusion, the results confirm our earlier finding that AII modulates responses to α agonists irrespective of the α-adrenoceptor subtype that is activated. Removal of endogenous AII inhibits whilst addition of exogenous AII facilitates α-adrenoceptor mediated pressor responses. AII may therefore be an important modulator of all α-mediated pressor responses in vascular smooth muscle.

TLG is supported by an S.E.R.C (CASE) Studentship in collaboration with the MRC Blood Pressure Unit, Western Infirmary, Glasgow.

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WHAT IS A SUITABLE O_2 TENSION FOR SMOOTH MUSCLE IN VITRO?

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Isolated tissues are often maintained in saline equilibrated, at atmospheric pressure, with a gas mixture of high oxygen content such as 100%, or, where CO_2 / bicarbonate buffering is employed, 95%. This produces PO_2 values of 300 to 650 mm Hg according to the efficiency of equilibration. The objective is to avoid a hypoxic core within the tissue; it is assumed that a high PO_2 will do no harm. This practice is not universal. Tyrode's solution bubbled with room air, has atmospheric PO_2 values (approx. 150 mm Hg) yet provides viable conditions even for thick preparations such as rabbit intestine. Those interested in the effects of hypoxia on smooth muscle routinely employ a bubbling mixture containing 16% oxygen for their **normal** PO_2 (usually 120-100 mm Hg) and reduce this further to produce hypoxia (Detar & Bohr, 1968; Ebeigbe & Jennett, 1978). Little attention has, however, been paid to the difference between normoxia, e.g. 16%, and hyperoxia, e.g. >90%.

We now report a comparison of the contractile responses of three smooth muscle preparations to noradrenaline (NA: 1nM-0.1mM) or to field stimulation of the intramural nerves (single supramaximal stimuli, 0.5ms), in Krebs' bicarbonate saline at 37°C equilibrated with 95% O_2 : 5% CO_2 or 16% O_2 : 5% CO_2 : 79% N_2. All tissues were obtained from male Wistar rats 250-350g. Isometric contractions were recorded from anococcygeus, and longitudinal strips of portal vein; tail artery was perfused with Krebs' at 4ml min^{-1} and perfusion pressure was recorded.

NA induced concentration-related contractions of smooth muscle in each preparation at each gas tension: these were α_1-adrenoceptor mediated responses; at the different gas tensions, pD_2 values, susceptibility to prazosin and resistance to the α_2 antagonist rauwolscine (except at high concentrations known to block α_1) were similar. However the time courses of the contractions changed with O_2 tension. At 16%, contraction was biphasic with most NA concentrations: an early rapid but transient component was followed by a slower, maintained component, normally of smaller size. At 95% the response was more monotonic. In anococcygeus, portal vein and tail artery, this change to a monotonic response in hyperoxia resulted both from a diminution in the rate of rise of the first component and an increase in the height of the second component. The nerve-induced contractions were not substantially different in anococcygeus between the two gas tensions.

Hyperoxia also alters responsiveness of vascular smooth muscle *in vivo* in the pithed rat: with ventilation on oxygen, α_1-mediated pressor responses were enhanced compared with those under normoxia (Grant et al, 1984). It is concluded that exposure to high PO_2 (i) makes little difference to pharmacological assessment of agonist-antagonist interaction at α_1-adrenoceptors, (ii) attenuates the initial rise in tension to NA and increases the maximal maintained contraction, suggesting that it alters the response beyond the receptor. With thin preparations it is suggested that physiological PO_2 is appropriate.

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VASCULAR PERMEABILITY ACTIONS OF KININS

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Bradykinin (BK) and related kinins are some of the most effective permeability increasing factors in most species (Wilhelm,1973). It has been suggested that this response to kinins is mediated via activation of a B_2-receptor (Regoli & Barabe, 1980) at least in the rabbit (Marceau et al,1981) since the selective B_1 receptor agonist, des-Arg^9-BK was inactive and the selective competitive B_1 receptor antagonist, des-Arg^9-Leu^8-BK did not antagonise the action of BK in this species. Chipens et al, (1981) have demonstrated that a cyclic analogue of BK, ε-cyclo-(Lys^1-Gly^6)-BK (Cyclo-BK) has low potency compared to BK in guinea-pig skin, and Whalley et al, (1984) found that the modified kinin fragment,H-D-Pro-Phe-Arg-pNA (S2302) was almost equipotent with BK in the rat paw oedema test. This study compares the effect of BK, Lysyl-BK (L-BK), Methionyl-Lysyl-BK (M-L-BK), des-Arg^9-BK, Cyclo-BK and S2302 in various models of vascular permeability.

Rat paw oedema was determined as described previously (Whalley et al, 1984) and vascular permeability in the skin of normal and rheumatoid arthritic (R.A.) rats, normal guinea-pigs and rabbits was assessed using the Evans Blue method described by Spector (1958). R.A. was induced in rats by the subplantar injection of 0.1 ml of a 10mg ml^{-1} suspension of dead tubercle baccilli in arachis oil 21 days earlier. From dose response curves to each kinin, the mean dose (nmoles) to produce a 15% (ED_{15}) increase in rat paw volume (20 min after injection) or the mean dose (nmoles) to give a 10 mm diameter leakage site (ED_{10}) in the skin (30 min after injection) were determined and the results are shown in the table below. BK was assigned a potency = 100 and the relative mean potency (P.R.) of each kinin to BK was determined. All animals were pretreated with captopril, 1mg kg^{-1}.n=6-20.

	Rat Paw ED_{15}	P.R.	Rat Skin ED_{10}	P.R.	Rat Skin R.A. ED_{10}	P.R.	G-Pig Skin ED_{10}	P.R.	Rabbit Skin ED_{10}	P.R.
BK	2.9 [a]	100	1.3	100	2.3	100	0.9	100	0.8	100
L-BK	5.6 [a]	52	5.6	23	3.1	74	0.7	128	0.6	133
M-L-BK	7.3 [a]	40	4.9	27	6.8	34			0.7	114
des-Arg^9-BK	1000 [a]	0.003	280	0.5	234	0.9	>1000	-	>1000	-
S-2302	7.0 [a]	41	5.2	25	4.4	52	>1000	-	>1000	-
CYCLO-BK	0.001	2.9×10^5	0.012	1.1×10^4	0.005	4.6×10^4	>100	-	>100	-

a) data from Whalley et al,(1984).

The low potency of des Arg^9-BK in all models suggests that activation of a B_1- receptor is not involved.Cyclo-BK and S2302 were ineffective in guinea-pig and rabbit skin but cyclo-BK was extremely potent in all rat models. The different relative potencies of the kinins may reflect a difference in receptors mediating responses in the rat compared to the guinea-pig and rabbit.

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COMPARISON OF DOSE-RESPONSE CURVES FOR FULL AND PARTIAL AGONISTS

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If two agonists, A and P, acting at the same receptor are both full agonists on a given tissue their potency ratio is a constant equal to $(e_A.K_P)/(e_P.K_A)$ where e = efficacy (Stephenson, 1956) and K = dissociation constant. If one or both of the agonists behave as a partial agonist their potency ratio is not a constant. A value R can however be estimated that is equal to $e_A.K_P/e_P.K_A$. R can therefore be used to compare the activity of agonists on different tissues irrespective of whether they are both full agonists or not. If the receptor type is the same, R should be constant regardless of the receptor reserve.

If agonist A has a higher maximum response than agonist P, and if [A] and [P] are equi-effective concentrations of the two agonists, then R is equal to the slope of the double reciprocal plot, 1/[A] against 1/[P] (Kenakin, 1984).

Alternatively R is equal to [P]/[A] minus [P]/[A*] as shown in Figure 1, where [A*] is the concentration of agonist A that produces the same response as the maximum produced by agonist P.

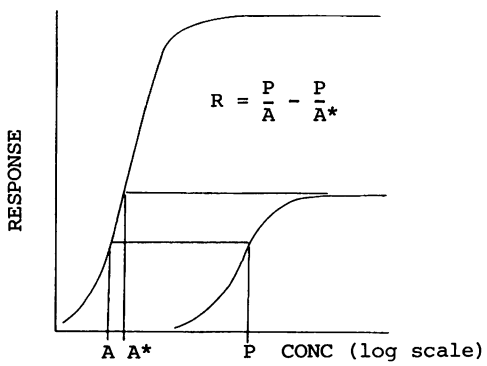

Figure 1

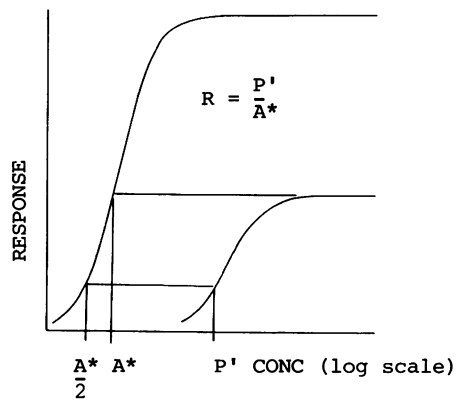

Figure 2

R is also equal to [P']/[A*] where [P'] is the concentration of agonist P that produces the same response as [A*]/2 of agonist A (Roberts, 1984). This is shown in Figure 2.

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ENHANCED K^+ RELEASE IN RAT PAROTID SLICES FOLLOWING CHRONIC PARASYMPATHETIC DENERVATION

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Parasympathetic denervation in the rat parotid gland, by surgical ablation of the auriculo-temporal nerve, causes a marked supersensitivity to cholinergic muscarinic agonists (Ekström, 1980). Previously, this supersensitivity has been examined in the anaesthetised rat by cannulating the parotid duct and measuring the volume of saliva produced in response to agonist administration but the composition of the secretion has not been investigated. In the present paper we report that supersensitivity can be observed in vitro as an increase in K^+ efflux (using ^{86}Rb as tracer) from the cells, offering a more convenient system with which to analyse the cellular mechanisms of denervation supersensitivity.

Unilateral section of the auriculo-temporal nerve was performed under pentobarbitone anaesthesia three weeks prior to the animal being killed by cervical dislocation. Both the denervated and contralateral control glands were removed, cleaned, trimmed of fat and then incubated in Krebs bicarbonate buffered saline containing 3-5 μCi/ml of ^{86}Rb for 30 min. Approximately 25 mg of tissue was then transferred to a flow chamber (0.3 ml volume, 6 ml/min) and the perfusate collected at 1 min intervals and the ^{86}Rb released counted. The radioactivity remaining in the tissue at the end of the experiment was determined and the activity in each sample expressed as a fraction of the total present (Putney, 1976, Gallagher, 1982). Drugs were applied for 5 min.

Carbachol and phenylephrine both produced a dose dependent increase in ^{86}Rb release which was inhibited by atropine and phentolamine respectively. In tissues which had been denervated three weeks previously, maximum response was unaltered but responses to lower doses were significantly increased (Table 1). The contribution of Na^+, Cl^- and Ca^{++} to this response can be investigated using this model.

Table 1

Carbachol (M)	^{86}Rb efflux Denervated	^{86}Rb efflux Contralateral Control	(P)
10^{-7}	19.3 ± 2.4 (n=7)	9.14 ± 1.52 (n=7)	< 0.01
3×10^{-7}	43.9 ± 10.1 (n=9)	18.1 ± 3.5 (n=9)	< 0.05
10^{-6}	86.1 ± 17.3 (n=13)	43.2 ± 6.3 (n=13)	< 0.05
3×10^{-6}	87.6 ± 9.4 (n=9)	59.1 ± 7.2 (n=9)	< 0.05
10^{-5}	101.1 ± 13.9 (n=17)	64.5 ± 9.2 (n=17)	< 0.05
10^{-4}	119.0 ± 19.0 (n=19)	115.0 ± 10.7 (n=17)	n.s.

All values are expressed as mean ± s.e.m. of (n) determinations.

This work was supported by the Cystic Fibrosis Research Trust.

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ANTAGONISM BY ATROPINE AND GLYCOPYRROLATE OF METOCLOPRAMIDE-FACILITATED GASTRIC EMPTYING

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The ability of metoclopramide to increase lower oesophageal sphincter tone and to facilitate gastric emptying is used in the treatment of disorders of the gastrointestinal tract and, prior to anaesthesia, to prevent gastro-oesophageal reflux. However, the latter use of metoclopramide might be compromised by the simultaneous use of atropine which can reduce lower oesophageal sphincter pressure and reduce gastric emptying (Brock-Utne et al, 1977; Costall et al, 1983). In an animal model we investigate the ability of atropine and glycopyrrolate to modify the action of metoclopramide to facilitate gastric emptying.

Dunkin-Hartley guinea-pigs (450-550g) were allowed free access to food and water or were allowed water only for 14h before use. Some guinea-pigs were implanted with a chronically indwelling intracerebroventricular (ICV) injection guide cannula stereotaxically located at Ant. 6.9mm, Lat. -2.2mm (with reference to the Kopf stereotaxic frame) and 2.0mm below the dura to allow subsequent metoclopramide/vehicle injection into the ventricular system. Gastric emptying was measured using a non-invasive X-ray fluoroscopic technique to follow the passage of polystyrene coated barium sulphate spheroids from the stomach (expressed as % spheroids leaving the stomach at 1h, Costall et al, 1983). n = 4-6 for each treatment.

The peripheral administration of metoclopramide (1-10mg/kg i.p.) to guinea-pigs having a low basal level of gastric emptying caused by prior food withdrawal dose-dependently increased the rate of gastric emptying (by 200-600% compared to vehicle, $P<0.01$-0.001, Mann-Whitney U-test). However, in these animals, atropine (0.5mg/kg i.p.) and glycopyrrolate (0.2mg/kg i.p.) failed to reduce gastric emptying. Conversely, in fed animals, having a normal rate of gastric emptying, metoclopramide (0.1-10mg/kg i.p.) failed to significantly modify the gastric emptying rate whilst atropine (0.5mg/kg i.p.) and glycopyrrolate (0.02 and 0.2mg/kg i.p.) were shown to reduce the rate of emptying by 60 to 90% ($P<0.001$). Pretreatments with atropine (0.0005 to 0.5mg/kg i.p., 30 min) or glycopyrrolate (0.0005 to 0.2mg/kg i.p. 30 min)dose-dependently antagonised the facilitation of gastric emptying caused by metoclopramide (10mg/kg i.p.) (ED_{50} values for atropine or glycopyrrolate 0.002mg/kg i.p.).

ICV administered metoclopramide (40-200µg) caused a rapid increase in gastric emptying even in fed guinea-pigs (approx. 200%, $P<0.001$). Peripheral pretreatment (30 min) with atropine or glycopyrrolate (0.002-0.02mg/kg i.p.) dose-dependently antagonised the ability of ICV administered metoclopramide to facilitate gastric emptying, reducing the level of emptying to control values.

It is concluded that the ability of metoclopramide to facilitate gastric emptying is (a) dependent on the basal rate of gastric emptying and (b) can occur following peripheral and central administration where (c) the effects occurring from either route are potently antagonised by atropine and glycopyrrolate. The clinical preanaesthetic use of the anticholinergic agents concomitant with metoclopramide would thus be predicted to antagonise the ability of metoclopramide to facilitate gastric emptying.

This work was supported in part by the Medical Research Council.

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EFFECT OF RESERPINE AND COCAINE ON SENSITIVITY OF PRE- AND POST-JUNCTIONAL ADRENOCEPTORS IN RAT VAS DEFERENS

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Supersensitivity to the effects of transmitters and related substances is ascribed to a spread of receptors away from the nerve terminal area, at least for cholinergic nerves at the neuromuscular junction (Thesleff, 1960). However, the reasons for the increase in sensitivity of adrenergically innervated smooth muscle are not so clear (Westfall, 1981). This study investigates the effect of cocaine and reserpine treatment on the sensitivity of prejunctional as well as postjunctional adrenoceptors in the rat vas deferens.

Postjunctional sensitivity was examined by estimation of the ED 50 values for noradrenaline and methoxamine in both the prostatic and epididymal ends of the tissue. These values were re-estimated after acute reserpine treatment (5 mg/kg 18h preceding sacrifice) and also after the same dose administered 7 days preceding sacrifice. Prejunctional sensitivity was examined by estimating the ID_{50} value for xylazine's inhibition of the twitch response of the two portions of the vas to a single pulse of field stimulation.

POSTJUNCTIONAL SENSITIVITY					
ED_{50} of NA ($\times 10^{-6}$M)					
Control	Cocaine	Short Reserpine	Long Reserpine	Short Res + Cocaine	Long Res + Cocaine
Epididymal ends					
7.96 ±0.97	1.73 ±0.20	7.75 ±0.55	9.77 ±1.12	2.08 ±0.64	3.00 ±0.61
Prostatic ends					
24.4 ±6.7	3.45 ±0.58	14.0 ±3.8	1.90 ±0.26	6.49 ±2.02	0.30 ±0.14

PREJUNCTIONAL SENSITIVITY			
ID_{50} for xylazine			
Epididymal		Prostatic	
Control	Long Res	Control	Long Res
2.44 ±0.28	1.66 ±0.20	6.41 ±1.50	4.35 ±1.23

The results show that cocaine potentiates the effects of NA to a greater extent in the prostatic ends of the tissue, possibly due to the more dense innervation and thus greater facility for uptake. Acute administration of reserpine was without effect but in tissues from rats reserpinised 7 days prior to sacrifice, the sensitivity of the prostatic end increased by a factor of 12, while the epididymal end was unaffected. This may reflect an increase in receptor numbers in the prostatic end. The effect of cocaine was unaffected by the process of reserpinisation. Methoxamine, which is not subject to neuronal uptake, was not potentiated by cocaine treatment, but the ED 50 was decreased from 15.1 x 10^{-6}M to 3.29 x 10^{-6}M 7 days after reserpine treatment. This is also consistent with an increase in postjunctional receptor density. By contrast, the prejunctional receptors seem to be quite unaffected by 7 day reserpine treatment, the ED 50's being unchanged. At the moment no satisfacotry explanation for this finding is available.

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[^{3}H]-PRAZOSIN BINDING DURING ISCHAEMIA AND REPERFUSION IN THE GUINEA-PIG LANGENDORFF HEART

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α-Adrenoceptor blocking drugs have been shown in various models to reduce the incidence of ventricular arrhythmias which result from myocardial ischaemia and reperfusion (Sheridan, 1982). In the cat, an enhanced α-adrenergic sensitivity has been demonstrated during reperfusion (Sheridan et al. 1980) which has been associated with an increase in α-adrenoceptor number (Corr et al. 1981). The present study examines [^{3}H]prazosin binding in the guinea-pig heart during myocardial ischaemia and during reperfusion.

Langendorff guinea-pig hearts were perfused (5ml $g^{-1}min^{-1}$) with a Krebs-bicarbonate solution at 32°C and paced at 3.3Hz (twice threshold voltage, 3msec pulse-width). Following 20 min control perfusion, hearts were made ischaemic by reducing flow to 10% of control for 30 min, at the end of which, flow was rapidly returned to normal. Ventricular arrhythmias developed consistently during ischaemia (ventricular tachycardia (VT) 80%, ventricular fibrillation (VF) 20%) and during reperfusion (VT 95%, VF 80%). Phentolamine (5μM) significantly reduced the incidence of arrhythmias during ischaemia (14 and 0%) and during reperfusion (VT and VF 14%).

After 20 min ischaemia or 1 min reperfusion, times at which arrhythmias most consistently developed, hearts were freeze-clamped in liquid nitrogen. Crude ventricular membrane fractions were prepared and assayed for [^{3}H]prazosin binding at 32°C using phentolamine (10μM) to determine non-specific binding. Control experiments were performed with time-matched perfused hearts which were not made ischaemic.

Table 1. Dissociation constants (K_D) and maximum number of binding sites (B_M) for [^{3}H]prazosin binding to guinea-pig ventricular membranes.

	Ischaemic perfusion		Control perfusion	
Time	B_M(fmol mg^{-1} protein)	K_D(nM)	B_M(fmol mg^{-1})	K_D(nM)
Perfusion 0 min	-	-	18.0±1.5	0.49±0.10
Perfusion 20 min	-	-	16.4±1.7	0.48±0.08
Ischaemia 20 min	15.5±1.4	0.38±0.06	15.8±1.5	0.48±0.05
Reperfusion 1 min	19.3±2.0	0.42±0.04	20.1±0.6	0.38±0.09

B_M and K_D values were similar for fresh tissues (18.1±3.0fmol mg^{-1}, 0.48±0.08nM) and after freezing in liquid nitrogen (18.0±1.5fmol mg^{-1}, 0.49±0.10nM). There was no significant change in [^{3}H]prazosin binding during control perfusions (Table 1). There was also no difference in either B_M or K_D during ischaemia or reperfusion when compared with controls.

Thus, although α-adrenoceptors appear to be implicated in arrhythmogenesis, in the perfused guinea-pig heart, neither the number nor the affinity of α-adrenoceptors is altered during ischaemia or reperfusion.

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MECHANISM OF POSITIVE INOTROPIC ACTION OF TRIFLUOPERAZINE IN GUINEA-PIG VENTRICULAR MUSCLE

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Trifluoperazine (TFP), a phenothiazine antipsychotic agent, is a potent inhibitor of calmodulin activity (Levin & Weiss, 1979). Calmodulin may influence cardiac contractility by affecting a number of calcium-mediated processes including calcium-dependent activation of cyclic nucleotide phosphodiesterase and inhibition of adenylate cyclase (Walsh et al, 1980). We have previously reported (Clarke et al, 1985) that TFP exhibited positive inotropic activity in guinea-pig papillary muscle and that this effect was not observed in rat tissue. This study investigates the mechanism of action of TFP-induced positive inotropism and compares the effect of TFP with that of the calmodulin antagonist, W7, N-(6-aminohexyl)-5-chloro-1-napthalenesulphonamide (Hidaka et al, 1981).

Isometric tension was recorded from right ventricular papillary muscles from rat and guinea-pig continuously superfused with 6 mmol.litre^{-1} K^+ physiological salt solution as described previously (Clarke et al, 1985). The effects on contractility of concentrations of each compound were measured over 15 min periods.

In guinea-pig papillary muscle TFP at concentrations less than 10^{-5} mol.litre^{-1} decreased contractility. At 5 x 10^{-5} mol.litre^{-1} a marked positive inotropic effect was observed (500 - 600% of control). Subsequent superfusion with 10^{-4} mol.litre^{-1} TFP was negatively inotropic. However, direct administration of 10^{-4} mol.litre^{-1} TFP did produce a positive inotropic effect. In rat tissue TFP was a weak inhibitor of contractility (pIC_{50} = 4.83) and was not positively inotropic. The positive inotropic effects of TFP in guinea-pig tissue were not abolished by reserpine pretreatment (5 mg.kg^{-1} i.p. 24 h previously) and were not affected by the H1 antagonist, mepyramine (10^{-5} mol.litre^{-1}). However, the effect was markedly reduced by the H2 antagonist, cimetidine (10^{-5} mol.litre^{-1}). Histamine (10^{-6} - 10^{-4} mol.litre^{-1}) was positively inotropic in guinea-pig, but not rat, papillary muscle. This effect was competitively inhibited by cimetidine and was not affected by mepyramine. The calmodulin antagonist, W7 (10^{-7} - 10^{-4} mol.litre^{-1}), produced a concentration-dependent decrease in contractility in both guinea-pig (pIC_{50} = 4.64) and rat (pIC_{50} <4.00) papillary muscles. No positive inotropic effects were observed.

These data indicate that the positive inotropic effects of TFP are not calmodulin-mediated. The differences between direct administration and administration of increasing concentrations of TFP could be indicative of an indirect mechanism of action. TFP may therefore produce positive inotropic effects in guinea-pig papillary muscle by inducing a release of histamine which stimulates H2 receptors in this tissue. These results may alternatively be explained by a partial agonist effect of TFP at H2 receptors. The former conclusion is supported by a previous report of TFP-induced histamine release in mast cells (Alm, 1983).

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THE INTERACTION OF NALOXONE AND CAPTOPRIL ON THE CONTROL OF CATECHOLAMINE RELEASE FROM THE ADRENAL MEDULLA OF THE DOG

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Captopril inhibits the reflex release of adrenaline and noradrenaline from the adrenal medulla in response to a fall in arterial pressure, and also inhibits both the resting release of catecholamines (CA) and that evoked by electrical stimulation of the splanchnic nerve in anaesthetized dogs (MacLean & Ungar, 1984, 1985). After inhibition by captopril, all components of CA release can be restored by the infusion of either angiotensin II or of ACTH. In preliminary experiments we found that infusion of naloxone also restores release inhibited by captopril.

Naloxone in man attenuates the hypotensive action of captopril (Rubin et al, 1984), and it has been inferred that captopril acts partly by inhibiting the degradation of endogenous opioid peptides.

In seven dogs anaesthetized with pentobarbitone (30 mg kg^{-1}) the venous outflow of the left adrenal gland was collected for the estimation of CA by HPLC. In four of the dogs the left adrenal gland was denervated and the peripheral cut end of the splanchnic nerve stimulated supra-maximally at 10 pps. In three dogs the carotid bifurcations were vascularly isolated and perfused with arterial blood, and baroreceptor tests were performed by lowering the carotid sinus perfusion pressure from 120 to 80 mm Hg for 2 min.

In the dogs with denervated adrenal glands, naloxone (infused i.v. at 15 μg kg^{-1} min^{-1}) raised the mean resting CA output from 24 to 152 pmol kg^{-1} min^{-1}, and the mean increment in output during splanchnic nerve stimulation from 50 to 117 pmol kg^{-1} min^{-1}. Captopril (1 mg kg^{-1} i.v.) abolished the response to splanchnic nerve stimulation, and during a second infusion of naloxone the increment on stimulation was restored to 41 pmol kg^{-1} min^{-1}.

Similarly, in the dogs with carotid perfusion, naloxone raised the mean resting CA output from 29 to 202 pmol kg^{-1} min^{-1}, and the mean increment in output during baroreceptor tests from 42 to 180 pmol kg^{-1} min^{-1}.

These results are compatible with a model of feed-back inhibition of CA release by co-released opioid peptides. This mechanism could act in parallel with the modulation of CA release by angiotensin and by corticosteroids. It does not seem necessary, in order to explain the interaction with naloxone, to postulate an additional action of captopril on opioid metabolism.

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A LOW COST SYSTEM FOR ON-LINE ANALYSIS OF CARDIOVASCULAR DATA

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The measurement of several channels of an oscillograph recording together with the derivation of variables not measured directly is a laborious and time-consuming task. The attraction of having this data processed on-line by computer is obvious. However to perform this processing a mini-computer has been necessary (Algate et al, 1983, Chapple and Donoghue, 1985). Such a computer system is expensive and may be beyond the reach of many academic departments. A micro-computer system was therefore developed which reaches a reasonable compromise between cost and efficiency.

The system hardward consists of an Acorn BBC 'B' with a 6502 second processor. The computer is connected by the analog and user ports via an interface unit to the J6 outputs of a Grass 7D polygraph. The J6 output follows the standard ECG convention of 2v so that this system could be used with any polygraph following this convention.

The software was written largely in BASIC with only the code controlling the interface in assembly language. The program uses the integral analog to digital converter which has a cycle time of 10 ms, to sample each channel for a three second period. Having sampled the voltage output from each channel the data may be processed in two ways. Firstly, data from channels giving a constant output (i.e. from an instantaneous ratemeter) are averaged. Secondly, data from channels giving a fluctuating output (i.e. blood pressure) are scanned to identify peaks and troughs which are each averaged. Most cardiovascular variables may be measured using one of these processing methods. The resultant data may then be converted to appropriate units and manipulated as required to give mean blood pressure, stroke volume, peripheral resistance etc. The program is completely menu-driven and all the calibration factors as well as any derivative expression are stored on disc and accessed at the beginning of the experiment. Data is updated on the screen after every sample period and sent to the printer at user-defined intervals, which may be changed during the course of the experiment. This sample period is indicated by means of the polygraph remote event marker so that the computer printout can be easily matched to the oscillograph trace.

The greatest disadvantage of this system is that the sample periods for each channel are not coincident. This is particularly important if the maximum of eight channels are used (24s sample time) with rapidly changing variables. In addition, when a variable is derived from separate channels, these must be adjacent to minimise the effect of separation. The system has been used to measure blood pressure and heart rate and the correlation between computer and manually measured data is good.

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DEPENDENCE OF Q-A INTERVAL UPON DIASTOLIC BLOOD PRESSURE AS WELL AS INOTROPIC STATE

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ECG 'Q' to aortic inscisura interval (QA) is claimed as an index of inotropic state, independent of heart rate and b.p. (Alabaster and Henderson, 1982; Cambridge and Whiting, 1984). Theoretical considerations of left ventricular isovolumic pressure development indicate that 1) isovolumic contraction time (the significant component of QA as regards inotropy) is dependent upon both the rate of pressure development and the timing of its cessation as determined by diastolic b.p. and 2) the relationship of QA with dPLV/dt, a widely accepted index of contractile function, must be hyperbolic, not rectilinear (Metzger et al., 1970. We have examined relationships between QA and dPLV/dt in a variety of experimental situations including sinus arrhythmia, lung inflation and administration of nor-adrenaline, isoprenaline, verapamil and phenylephrine in chloralose anaesthetized intact dogs. Results confirm the disruptive influence of diastolic b.p. on the relationship between dPLV/dt and QA (figure 1, crosses, r=-0.055). However, QA may be used to derive an indirect estimate of dPLV/dt by correcting for diastolic b.p. using the expression: dPLV/dt = BPdiastolic/(QA-EMI), (figure 1, squares, r=0.868). 'EMI' is the sum of mechanical delays (e.g. excitation-contraction coupling, cardiac conduction and catheter delays). In non-invasive circumstances EMI (excluding catheter delays) may be estimated as the time between ECG 'Q' wave and the onset of the apex cardiogram systolic upstroke (Manolas et al., 1975).

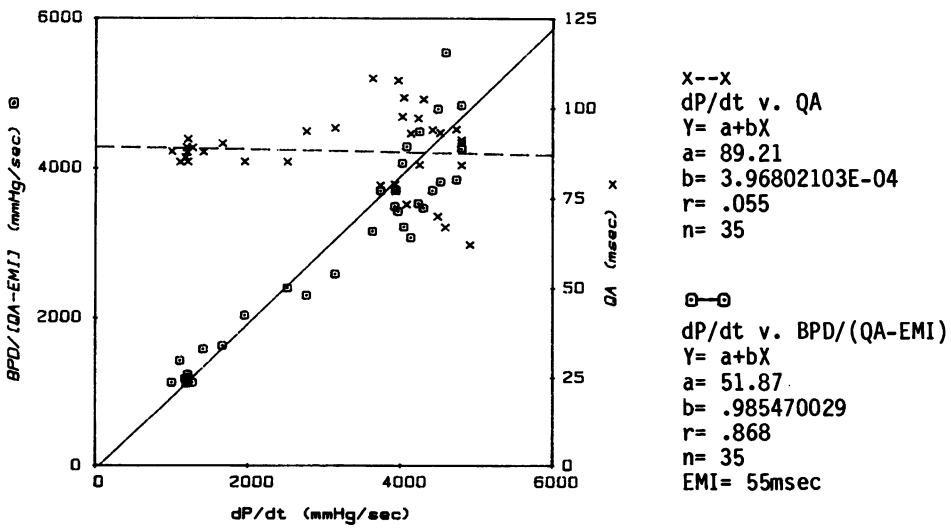

Figure 1. Lack of correlation between QA and dPLV/dt during lung inflation (x); diastolic b.p. compensation yields a significant relationship (⊡).

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EFFECTS OF PIRENZEPINE AND MDL 72222 ON CONTRACTIONS OF THE CAT NICTITATING MEMBRANES TO MUSCARINIC AGONISTS

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There is now considerable evidence for the heterogeneity of muscarinic receptors in both the central nervous system, (Hammer et al., 1980 ; Birdsall and Hulme, 1983). and in the periphery (Hammer and Giachetti, 1982 ; Kilbinger and Natziger, 1985).
In this study, we have compared the differential blocking effects of the selective M_1 receptor antagonist pirenzepine and the putative 5HT "M" receptor antagonist MDL 72222 (Azani et al., 1985) on M_1 or M_2 receptor-mediated nictitating membrane (N.M.) contractions, elicited by McN-A343 or carbachol.
Cats of either sex (2 - 4 kg) were anaesthetised with sodium pentobarbitone (45 mg/kg i.p.) and ventilated with room air using a Melsungen pump. Recordings of the N.M. contraction were made under a resting tension of 12 g. A lingual artery was cannulated to allow retrograde intraarterial (i.a.) injection of drugs, either to the blood supply of the N.M., or to the superior cervical ganglion (S.C.G.), by briefly occluding the external carotid artery.
Control nictitating membrane contractions were evoked by i.a. injections of McN-A343 (1 to 30 µg) to the S.C.G. A separate control dose-response curve was made to i.a. injection of carbachol (3 to 30 µg) to the N.M. Responses evoked by equieffective doses of McN-A343 (10 µg i.a.) or carbachol (10 µg i.a.) were repeated 10 min after the i.v. administration of pirenzepine (1, 3, 10 µg/kg i.v.), or MDL 72222 (0.03 - 1 mg i.a.). In a further series of experiments, the effects of pirenzepine and MDL 72222 were evaluated against the N.M. contractions evoked by frequency-response curves to preganglionic nerve stimulation (1 ms, supramaximal voltage, 0.1 to 25 Hz).
McN-A343, injected locally to the S.C.G., or carbachol, injected locally to the N.M., caused dose-dependent contractile responses of the N.M. McN-A343 was 3 times more potent than carbachol, and when injected locally to the N.M., did not cause any response.
Both pirenzepine (IC_{50} = 2.6 ± 0.5 µg/kg i.v.) and MDL 72222 (IC_{50} = 110 ± 20 µg i.a.) antagonized the N.M. responses evoked by McN-A343 (10 µg i.a.), but did not modify the contractions evoked by carbachol (10 µg i.a.). Neither pirenzepine, nor MDL 72222 antagonised the N.M. contractions evoked by preganglionic nerve stimulation, although MDL 72222 (IC_{50} = 490 ± 50 µg i.a.) but not pirenzepine antagonised the contraction of the N.M. evoked by 5HT (10 µg i.a.), injected to the S.C.G.
These results indicate that M_1 and M_2 receptor subtypes can be identified using the contractions of the N.M. in cats. M_1 receptors are responsible for muscarinic ganglionic depolarization, whereas the contractions of the smooth muscle of the N.M. are probably mediated through the activation of M_2 receptors.
In conclusion, pirenzepine is a very selective M_1 receptor antagonist, but the 5HT "M" receptor antagonist MDL 72222 may also have affinity for M_1 receptors in the cat S.C.G.

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EFFECT OF LAMOTRIGINE AND SOME KNOWN ANTICONVULSANT DRUGS ON VISUALLY-EVOKED AFTER-DISCHARGE IN THE CONSCIOUS RAT

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Lamotrigine [3,5-diamino-6-(2,3-dichlorophenyl)-1,2,4-triazine] is a novel, potent, anticonvulsant drug in the maximum electroshock (MES) test in mice and rats (Miller et al, 1984) and against electrically-induced after-discharge in the rat, dog and marmoset (Miller & Wheatley, 1985). In order to examine further the anticonvulsant profile of lamotrigine we have examined its effect on visually-evoked after-discharge (VEAD), a series of recurring (3-6Hz) waves or wave-spikes elicited by a single light flash and occurring after the late negative (N3) wave of the evoked potential. Abolition of the VEAD by anticonvulsant drugs is considered to be predictive of efficacy in absence seizures (King et al, 1980; Bigler E.D., 1977).

Male, hooded, PVG/Compton rats (200 to 250g) were implanted, under halothane anaesthesia, with three epidural stainless steel screw electrodes: the active electrode being positioned over the visual cortex, 7.0mm posterior to bregma, 3.0mm lateral to midline, with reference and ground electrodes located ipsilaterally at 1.0 and 12.0mm anterior to bregma respectively, and 1.0mm lateral to the midline. Following surgery there was a recovery and test acclimatisation period of at least fourteen days. On test days the rats were restrained with their eyes 6 inches from the stroboscope lamp of a photic stimulator (Devices) and stimulated at a frequency of 0.25Hz. EEG recordings of 1s. duration were recorded and 32 responses to the test stimuli were averaged and plotted on a chart recorder. In order to obtain consistent VEAD responses it was found necessary in the majority of experiments to give a low, non-convulsive, priming dose of leptazol (10 mg kg^{-1} i.p.) 20 min. prior to the test stimuli. Test drugs or control fluid (Celacol 0.25%) were given p.o. once weekly, using a randomised treatment schedule, 2 hours before recording. The end-point was the presence or absence of the VEAD and ED_{50} values to abolish VEAD were obtained by probit analysis (no. of doses per drug ≥ 4; no. of rats per dose ≥ 5).

Lamotrigine dose dependently inhibited the VEAD having similar ED_{50} values with or without leptazol pretreatment (ED_{50} = 3.2 and 3.5 mg kg^{-1}, respectively), as was also the case with diazepam (ED_{50} values = 1.7 and 1.9 mg kg^{-1}). Lamotrigine was approximately equipotent in both the VEAD and MES (ED_{50} = 5.6 mg kg^{-1}) tests. Phenytoin and carbamazepine, both effective in the MES test, were ineffective in the VEAD test at high multiples of MES ED_{50} values (x27 and x38 respectively). Conversely, ethosuximide, one of the principal drugs used in the treatment of absence seizures, was effective in the VEAD test (ED_{50} = 90.3 mg kg^{-1}) but inactive in the MES test at up to 960 mg kg^{-1}.

Other anticonvulsant tests indicated that lamotrigine had a profile similar to that of phenytoin and carbamazepine, the principal drugs for partial and generalised (tonic-clonic) seizures. The effectiveness of lamotrigine in the VEAD test suggests that the drug may also prove of value in the treatment of absence seizures. This suggestion remains to be confirmed clinically.

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CHARACTERISATION OF β-ADRENOCEPTORS ON CULTURED HUMAN EMBRYONIC LUNG CELLS

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We have recently described the β-adrenoceptor binding characteristics of cultured human cardiac cells (Brown et al, 1984). During long term culture and even at 6 h post mortem the number of β_1-adrenoceptor binding sites decreased compared with freshly autopsied tissue. Consequently, the % subtype (β_1:β_2) changed from 65:35 to 40:60. The present study investigates the β-adrenoceptors on cultured human embryonic lung cells (F2002 cell line). Data are compared with values obtained for guinea-pig lung and with those reported by Engel (1981) for biopsied human lung.

The F2002 cell line (Flow Laboratories) was cultured in a roller system in Eagles Basal Medium with 10% foetal calf serum. Scatchard binding assays were performed as described previously (Brown et al, 1984). The β-adrenoceptors on cultured cells demonstrated high affinity (Kd = 10 pmol) for the ligand (-)-[^{125}I]-iodocyanopindolol ([^{125}I]-cyp) and saturability (Bmax = 90 fmol.mg protein^{-1}). The characteristics of the β-adrenoceptor binding sites on cultured embryonic cells were assessed by examining the ability of betaxolol (β_1-adrenoceptor selective antagonist) and ICI 118,551 (β_2-adrenoceptor selective antagonist) to displace [^{125}I]-cyp binding. Both drugs produced biphasic displacement curves. These data were fitted to a two site model from which the following mean pKi values were determined (see Table 1; mean values with SE range, n = 3 - 6). Subtype % calculated from these data are also shown.

Table 1 pKi values for betaxolol and ICI 118, 551

	Cultured Human Lung		Biopsied Human Lung		Guinea-pig Lung	
	β_1	β_2	β_1	β_2	β_1	β_2
Betaxolol	**7.40**	**6.70**	**8.85**	**7.03**	**7.83**	**6.95**
	7.70-7.22	7.00-6.39	8.92-8.80	7.04-7.02	7.86-7.80	6.99-6.91
ICI 118,551	**5.70**	**8.51**	-	-	**6.46**	**8.24**
	5.92-5.55	8.60-8.43			6.52-6.40	8.30-8.18
% β_1:β_2	**20 : 80**		**30 : 70**		**20 : 80**	

Data for biopsied human lung are taken from Engel et al (1981).

The results indicate that the β-adrenoceptors on this human embryonic cell lines are mainly of the β_2-adrenoceptor subtype. Similar biphasic displacement curves for betaxolol and ICI 118,551 were also seen with guinea-pig lung membranes. All tissues have comparable subtype proportions which are similar to the 15:85% reported by Carswell & Nahorski (1983) for guinea-pig airways. These data suggest that in contrast to the β_1-adrenoceptors on cultured heart cells, β_2-adrenoceptors on human lung cells are maintained on long term culture and the cell line provides a useful source of tissue for the study of human β_2-adrenoceptors.

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CHLORDIAZEPOXIDE INCREASES TOLERANCE TO THE RESPIRATORY DEPRESSANT EFFECTS OF MORPHINE IN THE MOUSE

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The development of tolerance to very large doses of diamorphine (19.2 g daily) has been reported in a young patient with adult respiratory distress syndrome (McDonald et al, 1985). The suggestion was made that this may have been associated with the concurrent administration of benzodiazepines. This is the report of preliminary studies involving such interactions in mice. Mice can be made tolerant to the effects of morphine and other narcotic analgesics by a number of techniques involving daily injections, or implantation of pellets which release the active substance slowly. These techniques, however, are either time consuming or self limiting due to the encapsulation of the pellets. Oral self administration of morphine in rats is hampered due to the bitter taste of morphine, which tends to reduce the intake. In mice, however, the bitter taste does not seem to discourage water consumption, and the animals will readily consume water containing up to 600 mg/l.

Respiration frequency was measured in mice using a monitoring device described by Crossland et al (1977). Respiratory depression was calculated by measuring the percentage change in frequency 30 min after the administration of morphine (10 mg/kg). The effects of this morphine challenge were estimated in mice which had been drinking the equivalent of 40 mg/kg morphine daily with or without the addition of chlordiazepoxide at 10, 20, or 40 mg/kg daily.

RESULTS

Treatment		Week No 1	2	3	4
Water	M	92 ± 9	58 ± 11	58 ± 6	59 ± 5
	F	77 ± 10	77 ± 11	62 ± 8	56 ± 5
Morphine Alone	M	39 ± 6	58 ± 9	40 ± 6	45 ± 4
	F	42 ± 5	58 ± 5	44 ± 6	50 ± 6
Morphine + Chlordiazepoxide	M	43 ± 5	37 ± 5	26 ± 4	28 ± 3
	F	47 ± 6	44 ± 5	20 ± 3	32 ± 6

Values are % respiratory depression with standard errors. Each value is the mean of 10 mice.

The results would indicate that oral administration of morphine does make mice tolerant to the respiratory depressant effects of a subcutaneous injection of morphine. It is also apparent that the concurrent administration of chlordiazepoxide seems to increase the development of tolerance. This may be of some clinical importance when opiates and benzodiazepine are administered concurrently to aid synchronisation of patients' spontaneous respiration with positive pressure ventilators.

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ACUTE INTERACTIONS OF ETHANOL AND FLURAZEPAM

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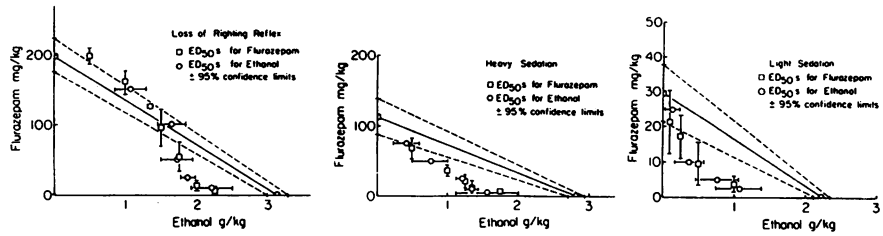
Combinations of sedative drugs and ethanol are frequently involved in deaths from suicide and accident. Clinical or experimental data available concerning lethality, or effects on motor co-ordination and alertness of combinations of large doses of ethanol and sedative drugs are rarely sufficiently comprehensive to allow construction of dose-response curves, or to determine if potentiation (synergism) occurs. We have studied the effects of a large number of combinations of flurazepam and ethanol in mice. By comparison of known doses for common end-points (e.g. lethality or hypnosis) of the individual drugs, these data should have predictive value for outcomes in cases of human overdose.

Groups of 6-24 male ICR mice were used for each dose combination. Drugs were injected i.p. into 2 separate sites; if possible, volumes were limited to 0.05 ml/g weight. The following acute endpoints were observed light sedation, heavy sedation (hypnosis), loss of righting reflex (motor co-ordination), and anaesthesia (all within 1 h of injection), and death (within 18 h). ED_{50}s for each drug alone and in combination with several doses of the other were determined by probit analysis. These data were plotted as 50%-response isobolograms for each end-point (Reiffenstein & Mah, 1984).

Figure 1 Isobolograms



Below 3 g/kg, ethanol does not affect flurazepam lethality (LD_{50}≈300 mg/kg) nor does less than 250 mg/kg flurazepam greatly affect the ethanol LD_{50} (≈4 g/kg). Greater doses are additive. The drugs were less than additive for anaesthesia. Effects on motor co-ordination (Fig. 1, left) are additive for high flurazepam - low ethanol combinations, but synergistic for ethanol above 1.5 g/kg. More than 0.8 g/kg ethanol and low doses of flurazepam were synergistic (Fig. 1, centre) in causing heavy sedation (no response to handling). Ethanol and flurazepam were synergistic for light sedation (Fig. 1, right).

Assuming that the human hypnotic dose of flurazepam (30 mg) is equivalent to a mouse dose of 80-100 mg/kg, it can be predicted that for at least 2 days after a single dose of flurazepam, low to moderate doses of ethanol will have a greatly exaggerated effect on motor co-ordination and alertness.

Supported by the Distilled Spirits Council of the United States, Inc.
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A SINGLE DOSE OF THE BENZODIAZEPINE RECEPTOR LIGAND FG 7142 CAUSES PROLONGED β-ADRENOCEPTOR UP-REGULATION IN MOUSE BRAIN

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We have shown recently that repeated administration of the benzodiazepine receptor ligand FG 7142 causes increases in the number of α^2 and β-adrenoceptors in mouse cerebral cortex (+ 25% in each case)(Little et al, 1985). FG 7142 has a high affinity for benzodiazepine receptors, but has effects opposite to the benzodiazepines, being proconvulsant and anxiogenic (Dorow et al, 1983, Little et al, 1984). We carried out the present study on the effects of a single dose of this compound for comparison with the effects of repeated administration. The dose of FG 7142 we have used was found previously to lower seizure threshold to pentylenetetrazol, but these effects had disappeared by 1½ hours after the I.P. injection of FG 7142 (Little et al, 1984). However, we now report that changes in adrenoceptor binding in mouse cortex are found one week after this single dose.

Male CDI mice were injected I.P. with FG 7142, 40 $mg.kg^{-1}$; controls received a vehicle injection (Tween 80, 1 drop in 10 ml. distilled water). All animals were killed seven days later and radioligand binding carried out on cerebral cortices, as described previously (Jefferys & Stanford, 1984). Three batches of animals were studied on separate occasions (n = 5-10 in each treatment group). The pooled results from the three batches are shown in the following table:

	^{3}H-Clonidine (α_2)		^{3}H-Dihydroalprenolol (β)	
	Kd	B_{max}	Kd	B_{max}
Control	0.58 ± 0.1	131 ± 9	0.52 ± 0.1	151 ± 15
FG 7142	0.46 ± 0.1	138 ± 9	0.59 ± 0.1	212 ± 24*
% paired control	97 ± 11	113 ± 11	114 ± 19	159 ± 20

Values show: mean ± s.e.m., B_{max} (p.Moles g^{-1}prot.) and Kd (nM).
* $p<0.05$ (Mann-Whitney U-test). n = 23 (each group).

Each batch showed similar changes in β-adrenoceptor binding after FG 7142; the increase in B_{max} was statistically significant in each case (percentages: 169%, 159% and 142%). The rise in α_2-adrenoceptor B_{max} was less consistent and not significant (92%, 123%, 137%). There were no changes in Kd for either binding site.

These results are surprising since the change in β-adrenoceptor B_{max} was greater than that reported previously after repeated administration of FG 7142. Furthermore, this is the first time that persistent changes in receptor binding have been shown after a single dose of an apparently short-acting drug. Although benzodiazepines are known to alter noradrenaline transmission, little is known of the effects of β-carbolines, such as FG 7142, on monoaminergic neurones. We are currently investigating possible explanations for the above changes.

We thank Ferrosan (Denmark) for supplying FG 7142.

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THRESHOLD EFFECTS: A SOURCE OF ERROR IN pA_2 DETERMINATIONS

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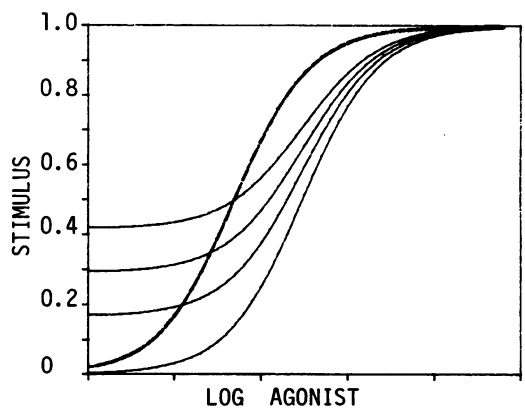
The affinity of an antagonist in an isolated tissue preparation can be readily assessed by classical methods (Arunlakshana and Schild, 1959). However, circumstances can arise where a high proportion of receptors are required to be activated before any response is detectable. Under such conditions partial agonists are indistinguishable from pure antagonists. An example of a tissue exhibiting this threshold effect is the field-stimulated rat isolated vas deferens preparation. This model appears to possess opioid receptors of the μ-type and, whilst it responds to the μ-agonist [D-Ala^2,MePhe^4,Gly-ol^5]-enkephalin, the μ-partial agonist morphine behaves as an antagonist (Henderson et al., 1982; Smith and Rance, 1983).

In such preparations, whilst the partial agonist may not itself produce a response, the stimulus generated by the partial agonist still contributes to the overall effect of agonist/partial agonist combinations according to the equation:

$$s = \frac{A}{A + (K_A \times (1 + \frac{P}{K_P}))} + \frac{P}{P + (K_P \times (1 + \frac{A}{K_A}))} . I$$

where s = stimulus (i.e. fraction of receptors activated)
A and K_A are the concentration and affinity of the agonist
P and K_P are the concentration and affinity of the partial agonist
I is the intrinsic activity of the partial agonist

Fig 1 illustrates theoretical concentration: stimulus curves for the interaction of an agonist with partial agonists of equal affinities but with intrinsic activities of 0 (pure antagonist), 0.2, 0.35 and 0.5. It is clear that at stimulus values between 0.5 and 0.9 (the range over which responses would be measured in a tissue with a high threshold) the dose ratios produced by the partial agonists are significantly smaller than that produced by a pure antagonist of equal affinity. Thus the application of classical methodology to such a situation would lead to an underestimate of the affinity of the partial agonist.



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SYNTHESIS AND ANTITUMOUR ACTIVITY OF ALKYLATING AGENTS RELATED TO A LOCAL ANAESTHETIC

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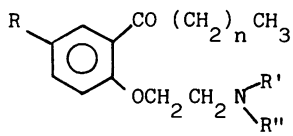
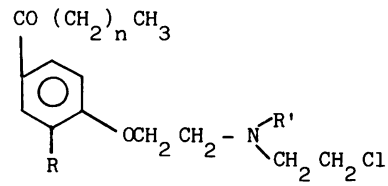
In our first paper (Andreani et al.,1983) we reported the synthesis and antitumour activity of two compounds (2, 3) related to the local anaesthetic ketocaine. The subsequent papers (Andreani et al.,1984,Andreani et al.,1983") concerned the analogues 4 - 13 while a new series (14 - 21) was published later (Andreani et al., 1984").

All compounds were tested in mice implanted i.p. with 10^6 Ehrlich ascites tumour cells. After 24h the animals were treated i.p. with a single dose of compound.

Para-compounds 14 - 21 show a behaviour analogous to that of ortho-compounds 2 - 13 as far as the introduction of a shorter chain is concerned. In fact shortening of the chain produces less active bifunctional antitumour agents while the influence on the monofunctional ones depends upon the presence of the nitro group : there is no influence on compounds 16, 17, while there is an increase in the activity of the corresponding nitro derivatives (the acetyl derivative 21 is more active than the propionyl derivative 20). A comparison between ortho-and para - -compounds shows that ortho-compounds are generally more active than the corresponding para-compounds : in particular compounds 4 and 10 showed potent antitumour activity (% T/C > 200).



1 n = 2; R = H; R' = R" = $CH(CH_3)_2$



14 - 21 n = 0 - 1

R = H, NO_2

2 - 13 n = 0 - 2; R = H, NO_2;

R' = CH_2CH_2Cl

R" = CH_2CH_2Cl, CH_2CH_2O-(R)-$CO(CH_2)_nCH_3$

R'=CH_2CH_2Cl, CH_2CH_2O-(R)-$CO(CH_2)_nCH_3$

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Research supported by Italian M.P.I.

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CALCIUM ANTAGONISTS AND SENSITIVITY TO ANTHRACYCLINES IN RESISTANT P388 LEUKAEMIA

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The biochemical and metabolic basis of acquired resistance to anthracyclines is not known, although selection of cells with altered membrane properties,resulting in reduced drug accumulation and retention, has been proposed as a mechanism of experimentally induced drug resistance.

Since it has been proposed that cell sensitivity to doxorubicin is dependent upon oxygen utilization, we have examined the relationship between doxorubicin sensitivity and oxygen consumption of P388 murine leukaemia cell line and of a doxorubicin-resistant subline. Oxygen utilization by doxorubicin-resistant subline was higher than that found in the sensitive line.

We observed that a variety of calcium antagonists (including channel blockers and intracellular antagonists : verapamil, trifluoperazine, dantrolene, TMB - 8) reduced oxygen consumption in Ehrlich ascites tumour cells. Moreover all these drugs enhanced the cytotoxic activity of doxorubicin in P388 murine leukaemia cell line and P388/DX resistant subline : the potentiating effect was marked in the resistant subline, but marginal for the sensitive line. This synergistic action of calcium antagonists was accompanied by reduction of oxygen consumption. Again, this phenomenon was more pronounced in the resistant cells.

These findings emphasize the inverse correlation between oxygen uptake and doxoru - bicin responsiveness.

Research supported by C.N.R. " Progetto Finalizzato Oncologia ".

RELATIONSHIP BETWEEN PROSTAGLANDIN D_2 AND CHOLINERGIC SYSTEM ON TRACHEAL SMOOTH MUSCLE

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The airways of asthmatic patients have long been known to be markedly sensitive to inhalation of a wide variety of different physical and chemical stimuly (1) . The mechanisms responsible for bronchial hyperreactivity are unknonwn, but the experimental evidence suggest that the disorder may stem from disturbance in mechanisms regulating airway smooth muscle tone rather than abnormality in the muscle itself (2). Furthermore airway hyperresponsiveness has been also associated to inflammation where arachidonic acid metabolites are deeply involved and may contribute to increase the activity of parasympathetic pathway. These basic considerations prompted us to investigate a possible interaction of Prostaglandin-D_2 (PGD_2)with the activity of the cholinergic system in guinea pig-tube preparation "in vitro". According to Coleman and Levy (3) the excitatory component of the elettrically induced byphasic response is mediated through a release of acethylcholine. PGD_2 induced a dose response potentiation of the cholinergic contraction of the trachea dissected from ovalbumine sensitized guinea pig. This phenomenon was less evident in non sensitized preparation, in fact only high concentrations of PGD_2 induced an increase in the cholinergic contractions.Indomethacin (3×10^{-6} M) added for 1 h. to the preparations obtained from normal and ovalbumin-sensitized guinea-pigs further increased the capacity of PGD_2 to augment cholinergic response. Furthermore since PGD_2 increases the contractile activity of acethylcholine on these preparations it is possible to imply that this prostaglandin may act on the muscarinic receptors; however an effect of PGD_2 on terminal cholinergic neurons cannot be excluded.

A physiopathological implication of these results may indicate that an inflammatory mediator of the eicosanoid system partecipates to the hyperreactivity to the airways strengthaning the cholinergic response.

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STEREOSELECTIVITY OF CHLORIDE CHANNEL STUDIED IN RAT SKELETAL MUSCLE BY CHIRAL ANALOGUES OF MYOTONIA INDUCERS

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An abnormally low chloride conductance (G_{Cl}) has been shown to be the basis for the repetitive firing seen in some forms of hereditary myotonia (Adrian and Bryant, 1974) and is the mechanism of myotonia produced by certain drugs such as clofibrate and its, in vivo, metabolite chlorophenoxyisobutyric acid (CPIB)(Conte-Camerino et al., 1984). Since in literature there are no evidences about a stereospecificity of the Cl^- channel, we studied the myotonic effects induced by chiral analogues of CPIB, prepared by substituting a methyl group with H and changing R (Figure 1).

The effects of those compounds were studied on the electrical parameters of rat extensor digitorum longus (EDL) muscle fibers, in vitro, with intracellular microelectrodes (Conte-Camerino et al., 1982). From the membrane resistances in normal and Cl^- free solutions we calculated the component conductances. As shown in Figure 1, G_{Cl} was drastically reduced by low concentrations of the (-) isomers of the compounds tested whereas the (+) isomers were virtually ineffective. Parallelly to G_{Cl} decrease we observed a dose-dependent increase of fibers excitability.

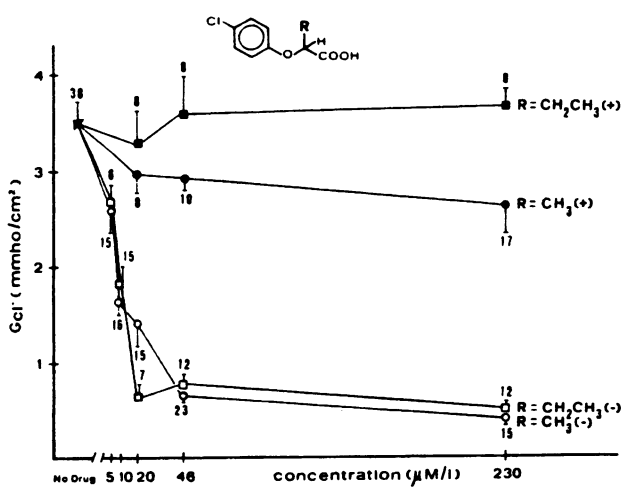

Figure 1 Membrane G_{Cl} of rat EDL muscle after incubation in various concentrations of CPIB chiral analogues. Each point represents the average ± s.e.m. of fibers (n° on the bars) from 3-5 muscles. The average G_{Cl} before addition of drug was taken as control (▶). $p < 0.01$, or less for each concentration of (-) isomers.

Our data demonstrate that there is a strong dependence between Cl^- ion flux in the channel and configuration of the tested compounds. In conclusion, these results, joined with the relatively low concentrations required to produce the described effects indicate a probable interaction with a specific receptor.

Acknowledgments: This research was supported by Italian CNR grants 83.00337204 and 84.0230804.

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EFFECTS OF VIDIAN NERVE STIMULATION AND RESECTION ON HISTAMINE CONTENT IN HUMAN NASAL SINUS MUCOSA

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Parasympathetic innervation of the nasal mucosa plays an important role in the pathogenesis of chronic hypertrophic non-allergic rhinitis (Golding-Wood, 1961). The Vidian nerve provides the main parasympathetic nervous supply to the nasal respiratory and maxillary sinus mucosa, and its electrical stimulation causes secretory and vasodilatatory effects in man (Rucci et al., 1984). We have previously demonstrated that vagal stimulation produces a histamine overflow in isolated, vagally innerved guinea-pig auricles (Blandina et al., 1983) and in isolated rat ileum in response to field stimulation (Blandina et al., 1984).

Aim of the present study is to evaluate the effect of Vidian nerve stimulation and resection on the histamine content and on the morphological pattern of nasal sinus mucosa.

The investigation is carried out in patients with chronic non-allergic rhinitis, undergoing the Vidian nerve resection. Before the neurectomy, the Vidian nerve was stimulated by means of two michrome wires with the following parameters: 0.8 ms, 6 Hz, 5 mA, for periods of 30, 60, 90 sec. Samples are taken from nasal sinus mucosa for histamine determination and microscopical observations before and after the stimulation period. The Vidian nerve stimulation causes a significant decrease in histamine content in the mucosa samples. On the microscopical observation, a significant variation in the glandular, stromal and vascular components are found. The changes indicate an enhanced secretory activity, an intensive vasodilatation and an intense degranulation of mast cells. The neurectomy of Vidian nerve resolves completely the clinical symptomatology while decreasing the mucosal histamine content even several months after resection.

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HUMAN VAS DEFERENS MOTILITY FOLLOWING IN VITRO AND IN VIVO ADMINISTRATION OF ATROPINE AND DIAZEPAM

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Work supported by "Insieme Contro La Droga Association" Genova,Italy

Human vas deferens motility induced by different agonists was studied by isolated organ tecnique.Results were presented .
Tissues showed two specific motility patterns of response to norepinephrine(NE) and dopamine(DA).In this work we have tested the influence in vivo and in vitro of preanaesthetic drugs(atropine and diazepam) on tissue motility induced by NE and DA.Tissues used were taken off during surgical interventions with different preanaesthetic and anaesthetic procedures. We could test NE and DA on different groups of tissues,obteined from patients that were:
- not treated with atropine and diazepam (group 1);
- treated with atropine and diazepam I.M.(group 2);
- treated with atropine and diazepam I.V.(group 3).
Furthermore we tested NE and DA on group 1 tissues pretreated in vitro with atropine or diazepam.

Results can be summarized as follows:
- organ motility was not influenced by I.M. administration of atropine and diazepam;there were no significative differences in response patterns between tissues from groups 1 and 2;
- I.V. administration of these drugs partially inhibited the response to NE, while response to DA was suppressed (group 3);
- atropine, directly preadministrated in the bath, showed a similiar effect as in vivo I.V. administration:responses to NE were globally decreased, and expecially tonic pattern is inhibited and responses to DA were completely suppressed;
- diazepam suppressed the tonic responses to NE and decreased the frequency of clonic responses induced by both NE and DA.

All these facts suggest two interesting questions. The first one concerns the pharmacokinetics aspects of I.V. versus I.M. administration in front of bio-phase and active concentration. The second problem concerns the mechanism of atropine and diazepam with the NE and DA postsynaptic receptorial systems.

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PROPERTIES OF PLASMA SH GROUPS IN EXPERIMENTAL INFLAMMATION IN RAT

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A proportionality between SH group reactivity (R) and concentration (C) of rat plasma and serum bovine albumin (BSA) in the SH-SS exchange reaction with 5-5' dithiobis (2-nitrobenzoic) acid (DTNB) was found. For this proportionality C and R/C ratio (Kr) seem to be appropriate parameters to characterize the plasma SH groups in biological studies.

No change of plasma SH reactivity was found by previous Authors in rat carrageenan paw edema (Butler et al.,1969). On the contrary an increased reactivity was observed in adjuvant arthritis.
We repeated the same experiments measuring R, C and Kr. A significant decrease of concentration, no change of reactivity and significant Kr increase were found in rat carrageenan paw edema. In arthritic rats plasma SH concentration was significantly decreased and the reactivity and Kr were significantly increased.
Then in both inflammation models the same correspondence was found, i.e. C decreased and Kr increased.

During inflammation electrophilic agents capable to attack protein SH groups are generated (Tappel, 1973).
Rats treated with electrophilic agents, as diethylmaleate (DEM) showed a decreased SH concentration and an increase of Kr. In vitro experiments with various electrophilic compounds confirmed the in vivo results and showed decreased concentration and increased Kr. In particular we observed that these agents have two distinct actions on the SH groups of BSA and on the rat plasma: an initial increased reactivity was followed by a decrease of SH concentration.

Similar events were found in inflammation experiments as in DEM treated rats. Therefore it seems likely that the change of plasma SH group properties during inflammation was caused by the action of electrophilic agents on plasma SH groups.

Table 1. Reactivity (R,uM/min), concentration (C,uM) and Kr (min^{-1}) of plasma SH groups in carrageenan paw edema and in adjuvant arthritic rat. M ± SD on 5 animals.

CONTROLS			PAW EDEMA			ARTHRITIS		
R	C	Kr	R	C	Kr	R	R	Kr
307^{a}	$231^{b,d}$	$1.33^{c,e}$	298	165^{d}	1.81^{e}	450^{a}	130^{b}	3.60^{c}
±40	±12	±.17	±27	±29	±.19	±50	±28	±.80

a, b, c, d, e significantly different P 0.05

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4-AMINOPYRIDINE AND GASTRIC SECRETION

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Aminopyridines (APs) facilitate chemical synaptic transmission at central, autonomic and neuromuscolar synapsis by a mechanism related to their property to exert a potent potassium channel blocking action in axonal membranes (Bowman & Savage, 1981). Among the APs, 4-Aminopyridine (4-AP) has been the most sudied in experimental and clinical trials but there is a lack of information concerning the biological actions of the drug on gastric secretion.

In the present research, the gastric secretory effect of 4-AP was studied in anaesthetized and unanaesthetized rats. Gastric secretion in urethane anaesthetized rats (200-300 g) was evaluated in stomach perfused rat preparation and un unanaesthetized animals using pylorus-ligated rats (Pendleton et al., 1981). Experiments on vagotomized and atropine-treated rats were also conducted.

Systemic administration of 4-AP (from 0.38 mg to 3.0 $mg.Kg^{-1}$) caused increase in rat gastric secretion comparable to that obtained with other stimulant drugs (H and ACh). At doses only slightly greater than the threshold dose symptoms indicating activation of both autonomic and motor nerves were observed. Pre-treatment of the rats with atropine (100 $ug.Kg^{-1}$ i.v.) or vagotomy caused a net reduction of the gastric acid secretion induced by 4-AP. Furthermore a wide variability and a noticeable delay in the onset of maximal response to 4-AP were observed in these animals. In unanaesthetized rats systemic injection of 4-AP up to 5 $mg.Kg^{-1}$ clearly reduced the rat gastric secretion indicating that the effect of the drug is dependent on the depressed rate of gastric output.

Owing to the high penetration of 4-AP from the blood into the nervous system (Lemeignan et al., 1984) from the present data it is possible to argue that 4-AP enhances gastric secretion by both central and peripheral mechanisms. In addition, the incompleteness of the cholinergic blockade suggests that the action of 4-AP on gastric secretion is exerted through the involvement of several organ systems all taking part to the secretory mechanism.

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