M-7 STIMULATES VASCULAR POSTSYNAPTIC β_2-ADRENOCEPTORS IN RATS

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The aminotetraline derivative, M-7, was recently shown to be a relatively selective agonist of postsynaptic α_2-adrenoceptors (Drew, 1980; Cavero et al., 1982), in addition to being a stimulant of dopamine receptors (Long et al., 1975). Since, like dopamine, to which they are chemically related, several members of this chemical series have a complex cardiovascular pharmacological profile, we have investigated whether M-7 also stimulates postsynaptic β_2-adrenoceptors.

Male rats (Sprague Dawley and Long Evans) weighing 200-250 g were anaesthetized with sodium pentobarbitone, artificially ventilated and pithed. They were prepared for arterial pressure and heart rate measurements. Dose-pressor responses to M-7 were determined by injecting each successive increasing cumulative dose after (within 20-30 sec) the previous one had given an apparent maximal effect. These curves were generated in rats pretreated 15 min earlier with either i.v. saline, yohimbine (0.3 mg/kg) and/or propranolol (0.75 mg/kg).

The vasopressor effects produced by M-7 were dose-related and not significantly affected by propranolol in both Sprague Dawley and Long Evans pithed rats. In yohimbine pretreated rats the dose-response curve to M-7 was shifted to the right and characterized by a two distinct components (Fig. 1). This shift was smaller and the biphasic aspect of the dose-response curve disappeared after pretreating the rats with propranolol. M-7 did not produce, in the doses studied, any significant change in heart rate.

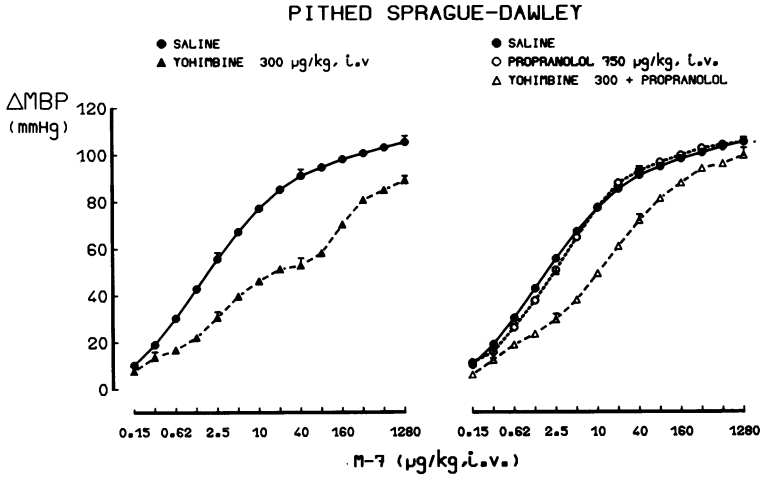

Fig. 1: Dose-pressor response curves to M-7 in rats (n=6-25/group) given various pretreatments.

These results indicate that M-7 stimulates vascular β_2-adrenoceptors. This effect, that becomes manifest after the α_2-adrenoceptor antagonist, yohimbine, should be blocked, where required, to allow the correct assessment of the relative selectivity of M-7 toward α_2-adrenoceptors.

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DIFFERENCES BETWEEN α-ADRENERGIC AND β-ADRENERGIC INOTROPIC EFFECTS IN GUINEA-PIG PAPILLARY MUSCLES

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As have been shown for rat ventricular myocardium, α- and β-adrenergic stimulation lead to qualitatively different inotropic responses (Skomedal et al, 1982). In order to see if these observations had more general applicability, we also studied electrically driven papillary muscles from the heart of another species - guinea pigs. The same method for isolation and mounting of the guinea pig papillary muscles was used as described for the rat papillary muscles (Skomedal et al, 1982). Isometric tension (T) and rate of rise and decline of tension (first derivative = T') were recorded. As the guinea pig myocardium has a force-frequency relationship which differs from that of the rat myocardium (Forester & Mainwood, 1974) some of the muscles were tested at a rate of 3 Hz in addition to the usual stimulation rate of 1 Hz. The guinea pig papillary muscles were exposed to: 1) 6.7×10^{-5} M phenylephrine in the presence of 5×10^{-6} M propranolol (α-adrenergic stimulation); 2) 9×10^{-8} M isoprenaline (β-adrenergic stimulation) and 3) 10^{-3} M N^6-2'-O-dibutyryl-cyclic AMP (DBcAMP) in the presence of 5×10^{-6} M propranolol in order to bypass the receptors. The inotropic response to β-adrenoceptor stimulation developed monophasically within 2 min after addition of agonist. At 1 Hz the contraction expressed as Tmax increased by 60.2 ± 17.3% compared to control value. The inotropic response to α-adrenoceptor stimulation showed a quite different time course: after a transient reduction in contractile force to subcontrol values (transient negative inotropic phase; Tmax was reduced by 15.2 ± 5.1% compared to control), there was a slowly developing positive inotropic phase which reached maximum 5-6 min after addition of the agonist. The contraction expressed as Tmax increased by 46.7 ± 6.5% compared to control value. Also at a stimulation rate of 3 Hz, there was an inotropic response after α-adrenoceptor stimulation which was qualitatively and quantitatively comparable to that observed at 1 Hz. Relaxation was expressed by the normalized measure, T'min/Tmax. This is an index which will compensate for an increase in T'min merely due to increase in Tmax without change in the duration of the contraction-relaxation cycle. After α-adrenoceptor stimulation this index did not change significantly from control value, indicating no selective increase in relaxation compared to contraction. After β-adrenoceptor stimulation this index increased significantly (15.0 ± 8.3%) when the response was fully developed, indicating a selective increase in relaxation compared to contraction. At 1 min after addition of the β-adrenoceptor agonist there was a transient reduction in this index to subcontrol values probably indicating that the selective increase in relaxation developed after a longer "lag phase" than did the increase in contraction (Tmax). DBcAMP mimicked qualitatively the response to the β-adrenoceptor stimulation, although the development of the response was rather slow. No transient reduction of T'min/Tmax was observed after addition of DBcAMP. This discrepancy between the β-adrenergic response and the DBcAMP response may be explained by the slow development of the DBcAMP response.

Thus, qualitative differences between the α-adrenergic and the β-adrenergic inotropic responses, respectively, were also demonstrated in guinea pig papillary muscle. The striking qualitative similarities between the β-adrenergic inotropic response and the DBcAMP inotropic response support the view that β-adrenoceptor stimulation is mediated by cyclic AMP, while α-adrenoceptor stimulation is cyclic AMP independent (Osnes & Øye, 1975).

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THE INCREASE OF MYOCARDIAL CONTRACTION AFTER THE β-AGONIST PRENALTEROL DEVELOPS FASTER THAN THE INCREASE OF RELAXATION

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β-Adrenergic stimulation increases both contraction and relaxation of cardiac muscle, the increase of the latter being the greater. We studied the time course of the response to the partial β-agonist prenalterol in isolated rat papillary muscle electrically driven at 1 Hz. We measured contraction and relaxation by recording maximal isometric tension (Tmax), maximal rate of tension rise (T'max), of tension decline (T'min) and maximal rate of transition from rise to decline of tension, i.e. maximal onset-rate of relaxation (T"min = minimum of the second derivative of tension). We also calculated the ratio T"min/T'max, the relaxation-onset index. This is a normalized measure for processes which selectively promote relaxation because it corrects for effects on T"min caused merely by a change in the contraction amplitude without change of the time course of the contraction-relaxation cycle. β-Adrenergic stimulation increased this index (Skomedal et al, 1982). The response to a submaximal dose of prenalterol (10^{-6} mol/l) levelled off after about 6-10 min. The time to half maximal response ($t_{\frac{1}{2}}$, in s) and the maximal values obtained (in per cent, control = 100%) for the different measures were (mean ± s.e. mean):

	Tmax	T'max	T'min	T"min	T"min/T'max
$t_{\frac{1}{2}}$	68±7	87±9	123±10	131±14	190±31
Max. value	122.7±3.3	129.1±3.5	149.6±5.5	160.3±6.8	125.9±2.4

Prenalterol thus differed from the previously reported time course of the response to a submaximal dose of the full agonist isoprenaline (Skomedal et al, 1982) in two ways. Firstly, all mechanical effects developed at a slower rate than was the case after isoprenaline. Secondly, the augmentation of the onset of relaxation (measured as T"min and T"min/T'max) evolved at a slower rate than contraction (measured as T'max and Tmax). With isoprenaline (9×10^{-8} mol/l) the augmentation of contraction and relaxation developed concomitantly with a $t_{\frac{1}{2}}$ of 30-35 s. Prenalterol thus revealed a time-related partial dissociation between the augmentation of contraction and relaxation. The explanation for these differences may be sought in the fact that isoprenaline is a full agonist while prenalterol is a partial agonist. In accordance with the spare receptor hypothesis, Hedberg and Mattson (1981) showed that isoprenaline had to occupy less than ten per cent of the β-adrenoceptors in order to evoke a maximal response in cat myocardium. Prenalterol, however, had to occupy almost all β-adrenoceptors present in myocardial tissue in order to induce its maximal response. Prenalterol may thus need a longer time to occupy the amount of receptors necessary for evoking the mechanical response. In addition, prenalterol only partially activated the adenylate cyclase coupled to the receptors occupied while isoprenaline caused a maximal activation (Hedberg & Mattson, 1981). cAMP may therefore reach functional important sites sooner after an activation of the β-adrenoceptors by isoprenaline than after prenalterol. This would probably be most pronounced for sites at a distance from the sarcolemma. As the increased onset-rate of relaxation after β-adrenergic stimulation probably is mediated by cAMP dependent phosphorylations of sarcoplasmic reticulum and troponin I (for ref. see Osnes et al, 1980, review), the prolongation of the time course for the mechanical effects might be specially pronounced for the onset-rate of relaxation. Prenalterol may be a useful tool in further elucidating the mechanisms of the β-adrenergic effect because of the time-related partial dissociation between the development of contraction and relaxation.

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THE AFFINITY OF AGONISTS FOR CARDIAC β-ADRENOCEPTORS INCREASES DURING HYPOTHERMIA-INDUCED SUPERSENSITIVITY

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Hypothermia induces supersensitivity to the positive inotropic and chronotropic responses of guinea-pig isolated atria to sympathomimetic amines which is believed to be relatively specific for the β-adrenoceptor (Broadley, 1980). We have used the irreversible β-adrenoceptor antagonist Ro 03-7894 to determine the dissociation constants (K_A) of isoprenaline and orciprenaline in right atrial (RA) rate, left atrial (LA) tension and papillary muscle (PM) tension at different temperatures.

The tissues were suspended in Krebs-bicarbonate solution gassed with 5% CO_2:oxygen and incubated with metanephrine (10^{-5}M) and U-0521 (10^{-4}M) throughout to inhibit extraneuronal uptake and COMT respectively. Left atria and papillary muscles were paced at $2H_z$ (threshold voltage + 5%, 5ms pulse width) and isometric tension recorded. Cumulative dose-response curves to isoprenaline or orciprenaline were constructed. The tissues were then incubated with Ro 03-7894 (7.6×10^{-4}M) for 30 min and after washout during 3h, the agonist was repeated. Experiments were performed at either 38 or 30°C. Pre-antagonist curves were corrected from control experiments performed identically but without antagonist.

Geometric mean (n>8) EC_{50} values were determined from uncorrected pre-antagonist dose-response curves and were compared by Student's t-test. The values for orciprenaline on RA (1.01(0.74-1.37)μM), LA (1.36(1.02-1.82)μM) and PM (2.16(1.46-3.20)μM) at 38°C were significantly greater ($P<0.01$) than the corresponding values of 0.37(0.25-0.55), 0.19(0.10-0.37) and 0.69(0.38-1.23)μM obtained at 30°C. Similarly, for isoprenaline the mean (n>8) EC_{50} values of 3.3(1.8-6.0) and 7.16(4.0-13.0)μM for RA and LA at 38°C were significantly larger ($P<0.001$) than the values at 30°C (RA, 0.38(0.2-0.71); LA, 0.31(0.15-0.65)μM). This demonstrates that supersensitivity occurred at the lower bath temperature in the three cardiac preparations.

Incubation with Ro 03-7894 depressed the maximum responses to both agonists, characteristic of irreversible antagonism, although at the lower temperature there was less depression as described previously (Broadley & Nicholson, 1980). Dissociation constants (K_A) were calculated from the graph of I/A against I/A^1, where A and A^1 are equieffective concentrations on the dose-response curves obtained before and after Ro 03-7894; K_A=(slope-1)/intercept (Furchgott, 1966). The mean (n>4) K_A values of orciprenaline on RA, LA and PM (8.42±2.45, 4.31±1.25 and 7.87±1.34 x 10^{-6}M respectively) at 38°C were all significantly larger ($P<0.05$) than the corresponding values obtained at 30°C (2.32±1.1, 0.96±0.47 and 2.98±1.17 x 10^{-6}M respectively), indicating a higher affinity for the β-adrenoceptor at the lower temperature. Similarly, the K_A values of isoprenaline on RA (3.77±1.45 x 10^{-8}M) and LA (2.8±0.69 x 10^{-8}M) were significantly greater ($P<0.05$) than the K_A values of 0.45±0.16 x 10^{-8}M and 0.57±0.62 x 10^{-8}M for RA and LA obtained at 30°C.

These results suggest that the hypothermia-induced supersensitivity in cardiac tissues is associated with an increase in the affinity of agonists for the β-adrenoceptor.

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PHARMACODYNAMIC EFFECTS OF NIMODIPINE IN THE ISOLATED SPONTANEOUSLY BEATING RABBIT HEART

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The myocardial effects of the calcium antagonist nimodipine were investigated in retrogradely perfused rabbit hearts (mean wet weight 6.36 g ± 0.32 SE). Nimodipine was infused into aorta at stepwise increasing rates, which established the concentration levels stated in table 1. Pharmacodynamic steady states were reached within 25 min at each step of constant rate infusion. The results are summarized in table 1.

Table 1 Nimodipine effects in isolated rabbit hearts (n = 11). Levels of significance: ^{o} $p < 0.05$, * $p < 0.01$ and ** $p < 0.001$.

Concentrations in ng ml^{-1}	1.1	2.7	3.4	5.8	9.3
Contraction amplitude, a % ± SE	84.8** ±2.7	67.7** ±5.2	53.3** ±4.2	39.9** ±3.1	27.4** ±2.6
Contraction rate, da/dt % ± SE	84.2 ±4.3	63.0** ±4.2	49.2** ±3.5	38.8** ±3.4	25.5** ±1.8
Oxygen consumption, O_2 % ± SE	98.2 ±1.7	93.3^{o} ±2.9	85.9** ±2.9	72.9** ±3.9	60.1** ±4.9
Ratio $(da/dt)/O_2$ with 95 % confidence limits	0.86** 0.77 0.97	0.67** 0.58 0.77	0.57** 0.49 0.66	0.53** 0.43 0.64	0.42** 0.36 0.50
Coronary flow rate, % ± SE	98.3 ±3.0	96.9 ±5.4	93.1 ±5.6	82.3* ±5.2	67.9** ±5.6
Heart beating rate, % ± SE	94.3** ±1.1	87.1** ±2.7	78.4** ±3.5	69.7** ±5.7	63.5** ±6.5
QT-interval, % ± SE	103.7^{o} ±1.3	107.0* ±1.9	114.8** ±2.4	124.2** ±3.3	133.3** ±3.4

The progressive negative (but reversible) effect on myocardial contractility was very pronounced, and judged from that, the potency of nimodipine is about 2.5 times that of nifedipine. The same relative potency was found for the negative effect on the ratio of contraction rate to oxygen consumption, which is a possible expression of myocardial efficiency '(Nielsen-Kudsk & Askholt, 1981)'.

The negative chronotropic effect was more pronounced and the relative potency about 10 times that of nifedipine. Dromotropic effect evaluated from ecg-recordings showed: (1) a significant increase ($p < 0.01$) in the PQ-interval only at the highest stated concentration of nimodipine, (2) no changes in the length of the QRS-complex, but (3) a progressive and significant increase in the QT-interval. Pronounced depressions of both atrial and ventricular automaticity, were caused by higher concentrations than those stated in table 1.

The slow development of the pharmacodynamic effects of nimodipine supports as for nifedipine the assumption of a gradually increasing myocardial accumulation probably related to an action on intracellular calcium binding additional to the blocking effect on calcium ion uptake over the sarcolemma membrane.

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EFFECTS OF AMRINONE ON TENSION RESPONSES AND CYCLIC NUCLEOTIDE LEVELS IN RABBIT DEPRESSED PAPILLARY MUSCLE

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It has been previously reported (Honerjäger et al 1981, Martorana et al 1982) that the positive inotropic effect of amrinone is consistent with a mechanism involving cyclic nucleotides. In our previous studies with amrinone, papillary muscles were driven at a submaximal frequency (0.4 Hz) in order to obtain a more readily measurable inotropic response. We have now extended our studies to examine the effects of amrinone on cyclic nucleotides and developed tension in other models of myocardial depression.

Papillary muscles from the right ventricle of male NZ white rabbits were suspended in Krebs-Henseliet solution (KHS) at 32°C and their electrically evoked contractions recorded by conventional methods. Depressed states of contractility in preparations stimulated at the optimal frequency (1.0 Hz) were induced by using KHS containing one-quarter Ca^{2+} (0.625 mM) or by adding sodium pentobarbitone (160 µg/ml) to normal KHS. Cyclic nucleotide levels were measured using standard assay kits (Radiochemical Centre, Amersham). The results are summarised in Table 1.

Table 1 Effects of amrinone on tension responses and cyclic nucleotide levels in rabbit depressed papillary muscles. ±s.e. mean (n > 4)

Depressant	Conc. Amrinone (µg/ml)	Tension (mg)	c-AMP (pmol/mg)	c-GMP (pmol/mg)
Sodium Pentobarbitone	Solvent control	211 ± 27	0.80 ± 0.08	0.014 ± 0.003
(1.0 Hz, 160 µg/ml)	100	337 ± 58	0.96 ± 0.08	0.019 ± 0.005
2.5 mM Ca^{2+}	250	387 ± 87	1.12 ± 0.05	0.027 ± 0.005
	500	728 ± 132	1.20 ± 0.07	0.022 ± 0.007
	1000	710 ± 128	1.49 ± 0.05	0.037 ± 0.005
		(r = 0.88)	(r = 0.97)	(r = 0.90)
0.625 mM Ca^{2+}	Solvent control	364 ± 47	0.60 ± 0.08	0.013 ± 0.005
(1.0 Hz)	100	368 ± 40	0.81 ± 0.08	0.023 ± 0.003
	500	637 ±109	1.27 ± 0.12	0.027 ± 0.006
	1000	1181 ± 97	1.46 ± 0.13	0.051 ± 0.008
		(r = 0.98)	(r = 0.96)	(r = 0.97)
2.5 mM Ca^{2+}	Solvent control	303 ± 60	0.74 ± 0.06	0.039 ± 0.009
(0.4 Hz)	100	314 ± 90	0.77 ± 0.08	0.036 ± 0.007
	500	882 ± 206	1.22 ± 0.09	0.079 ± 0.006
	1000	1250 ± 109	1.47 ± 0.14	0.102 ± 0.010
		(r = 0.98)	(r = 0.98)	(r = 0.97)

In the depressed papillary muscles amrinone induced concentration-related increases in developed tension and in the levels of both cyclic nucleotides. Correlation co-efficients for these effects are given at the foot of each column in Table 1. The data obtained at 0.4 Hz are from Martorana et al (1982) and are shown for comparative purposes.

These results substantiate our previous finding that in rabbit papillary muscles the positive inotropic effect of amrinone is closely linked to changes in cyclic nucleotides. Furthermore, the relative magnitude of the amrinone inotropic response is greater in depressed tissue and is independent of the means by which myocardial depression is induced.

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VASODILATOR AND INOTROPIC EFFECTS OF THE ANTIARRHYTHMIC DRUG MELPERONE

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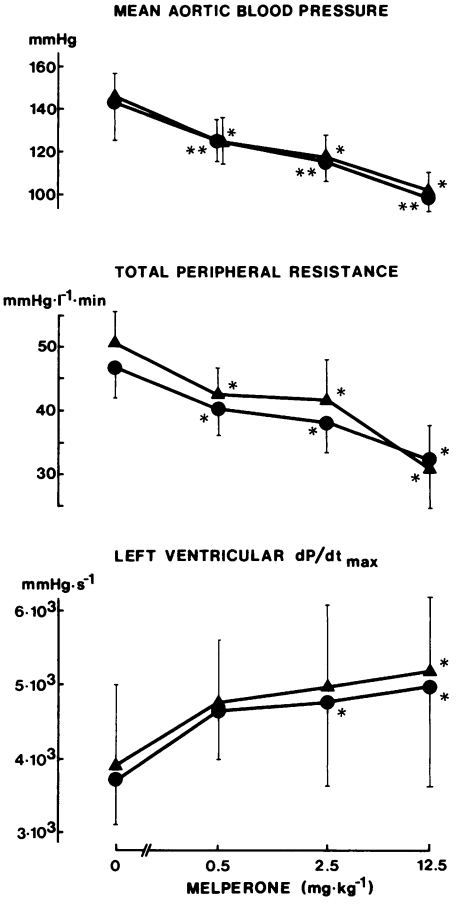
Since most antiarrhythmic drugs in current use may have cardiodepressive effects, we investigated the haemodynamic and direct mechanical effects of the new class III antiarrhythmic drug melperone.



In eight pentobarbital anaesthetized dogs, the effects of intravenous melperone 0.5, 2.5, and 12.5 mg/kg were tested. During spontaneous heart rate and atrial pacing we measured cardiac output (CO), mean aortic blood pressure (MAP), right (RV) and left (LV) ventricular pressures and LV dP/dt. In six RV papillary muscles isolated from cats and suspended in a muscle bath containing Krebs Henseleit solution, direct effects of melperone 10^{-8} to $5 \cdot 10^{-5}$ M were studied.

Administration of melperone decreased total peripheral resistance, MAP, RV and LV systolic and end-diastolic pressures, while CO was unchanged. Melperone increased LV dP/dt max. The contractile force (F) and dF/dt max of the isolated ventricular muscle preparations increased slightly with increasing concentrations of melperone, up to 10^{-5}M.

In conclusion, melperone has vasodilator and positive inotropic effects in addition to its class III antiarrhythmic effect.

Figure 1 Effects of melperone 0.5, 2.5 and 12.5 mg·kg^{-1} (log scale) on mean aortic blood pressure, total peripheral resistance and left ventricular dP/dt max at spontaneous heart rate (●) and when paced at 200 min^{-1} (▲). (Median ± 95% confidence interval, *: $p \leq 0.05$ **: $p \leq 0.01$).

CLASS III ANTIARRHYTHMIC ACTION IN EXPERIMENTAL ATRIAL FIBRILLATION AND FLUTTER IN DOGS: EFFECTS OF AMIODARONE AND MELPERONE

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Progress in the pharmacological treatment of atrial fibrillation and flutter has been achieved by the introduction of the class III antiarrhythmic drug amiodarone. In the present study we tested amiodarone and a new class III antiarrhythmic drug, melperone, in experimentally induced atrial fibrillation and flutter in pentobarbital anaesthetized dogs. By high rate stimulation in the right atrium, atrial fibrillation was induced in 4 and atrial flutter in 10 out of 22 dogs. Atrial flutter rate was 496±13 min^{-1} (median ± 95% confidence interval).

Both drugs converted the arrhythmias at doses from 2.5 to 10 $mg \cdot kg^{-1}$ and reduced the atrial flutter rate before conversion. Average ventricular rate during arrhythmias (261±16 min^{-1}) decreased after amiodarone and was unchanged or, in 3 out of 9 dogs, increased after melperone. The doses of amiodarone and melperone converting the arrhythmias, markedly increased atrial refractoriness. The effects of amiodarone on atrioventricular nodal conduction and refractoriness were variable, whereas melperone decreased atrioventricular nodal conduction time and refractoriness.

In conclusion, the study supports the concept that atrial flutter is due to circus movement where the flutter rate is dependent upon atrial refractoriness. The class III antiarrhythmic drugs amiodarone and melperone seem to be equally potent in converting atrial arrhythmias.

AUTONOMIC AND HORMONAL INFLUENCES ON THE SEVERITY OF CORONARY ARTERY LIGATION-INDUCED ARRHYTHMIAS IN ANAESTHETIZED RATS

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Coronary artery ligation in the anaesthetised male rat has recently been shown to be a useful model for the production of experimental cardiac arrhythmias and for the assessment of antiarrhythmic drug activity (Clark et al., 1980; Au et al., 1979). There is, however, a lack of knowledge about the mechanisms which underly these arrhythmias and the factors which influence their severity. The aim of the present study was, therefore, to examine this model in greater detail and in particular to investigate how it is modified by autonomic and hormonal influences. The results obtained are shown in table 1.

Table 1. The total number of extrasystoles (VE) and the duration of ventricular tachycardia (VT) and of ventricular fibrillation (VF) in animals surviving 30 minutes of coronary artery ligation. The percentage incidence of these arrhythmias is given in parentheses.

Group	n	V.E.	V.T. (s)	V.F. (s)
Male control	10	1171 ± 268 (100)	58 ± 18 (100)	26 ± 14 (50)
Vagotomised	9	*461 ± 258 (89)	39 ± 24 (56)	41 ± 15 (33)
β-blocked, atenolol 2mg/kg	10	872 ± 470 (100)	95 ± 63 (70)	55 ± 21 (20)
Vagotomised and β-blocked	10	*394 ± 150 (100)	*20 ± 10 (100)	0 (0)*
Females	18	*384 ± 72 (100)	20 ± 5 (94)	*8 ± 2 (33)
Pro-oestrus	10	*509 ± 146 (100)	32 ± 10 (70)	48 ± 22 (30)
Oestrus	15	560 ± 208 (100)	31 ± 16 (80)	41 ± 28 (30)
Dioestrus	10	*486 ± 168 (100)	38 ± 15 (80)	28 ± 3 (30)

Values expressed as mean ± s.e.m. were calculated only in those animals which exhibited that particular type of arrhythmia. n is the number of survivors in each group. Significance of difference from male control group * $P < 0.05$.

Bilateral section of the vagus nerves in the neck significantly reduced the number of extrasystoles. Blockade of the effects of sympathetic nerve stimulation by administration of a cardioselective β antagonist lacking membrane stabilising activity, atenolol, slightly reduced the number of extrasystoles and the incidence of fibrillation, although not significantly. A combination of vagotomy and β block abolished fibrillation and significantly reduced both the duration of tachycardia and the number of extrasystoles.

Female rats, approximately the same age as the males, exhibited less severe arrhythmias following coronary artery ligation. This reduced severity was not dependent upon the oestrus state of the females since rats in pro-oestrus, oestrus and dioestrus (identified by a vaginal smear on the day of the experiment) all exhibited a reduced number of extrasystoles upon ligation.

It is concluded that coronary artery ligation induced arrhythmias in the anaesthetised rat are partly dependent upon disturbances in the autonomic control of the heart since abolition of this control markedly suppressed them. Female rate, irrespective of the stage of their oestrus cycle, exhibited less severe arrhythmias following ligation.

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CATECHOLAMINE UPTAKE MECHANISMS: A STUDY IN GUINEA-PIG DEPOLARIZED ATRIAL PREPARATIONS

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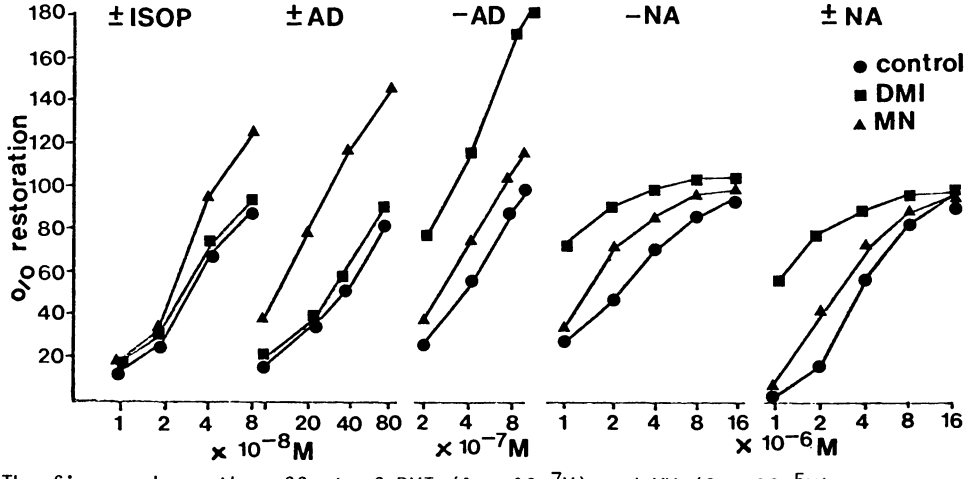
It has been reported that the extraneuronal uptake of catecholamines is poorly developed in guinea pig hearts (Goldie, 1976; Anning et al, 1979). It has also been suggested that this system lacks substrate stereochemical specificity, and is inhibited by extracellular potassium concentrations above 50 mM (Iversen, 1973). This study investigated the effects of inhibition of neuronal (U_1) and extraneuronal (U_2) uptake of noradrenaline (NA), adrenaline (AD) and isoprenaline (ISOP) by desipramine (DMI) and metanephrine (MN) in guinea pig depolarised atria.

Guinea pig isolated left atria were rendered unresponsive to punctate electrical stimulation at 0.5 Hz by immersion in Krebs solution containing 22 mM potassium. Contractile responses were restored by (±) - ISOP (1×10^{-7}M), (±) - AD (1×10^{-6}M), (-) - AD (1×10^{-6}M), (-) - NA (1.6×10^{-5}M) and (±) - NA (1.6×10^{-5}M). These responses were abolished by propranolol ($1\text{-}2 \times 10^{-7}$M), but not by phentolamine (1×10^{-6}M) indicating the involvement of β-adrenergic receptors. $MnCl_2$ (1×10^{-4}M) but not tetrodotoxin (1×10^{-6}M) also inhibited the responses, confirming that the inward depolarising current of the action potentials is carried by calcium ions (Pappano, 1970; Thyrum, 1974).



The figure shows the effect of DMI (1×10^{-7}M) and MN (2×10^{-5}M) on the size of the contractions restored by ISOP and NA. Restorations due to NA, both (±) and (-) forms were potentiated by DMI as expected, and were little affected by MN. ISOP restored contractions were potentiated by MN, but not by DMI, suggesting that a U_2 process does exist in guinea pig atria. With AD, it is interesting to note that while the (-) form is potentiated by DMI, indicating a U_1 process, the (±) form is potentiated almost exclusively by MN. This suggests that an extraneuronal uptake process does exist in guinea pig atria, at least in the presence of partial depolarisation. The reason for the preferential potentiation of the (±) form of adrenaline remains to be investigated.

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EFFECT OF OUABAIN ON TRITIUM EFFLUX IN THE RAT ISOLATED HEART PRELABELLED WITH (^{3}H)-NORADRENALINE

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The sympathetic nervous system seems to play a role in the ventricular arrhythmias induced by cardiac glycosides but whether cardiac glycosides increase or decrease catecholamines release from the peripheral organs is controversial (Gillis et al, 1978; Lathers & Roberts, 1980). It was of interest, therefore to examine the effect of arrhythmic concentrations of ouabain on the release of catecholamines from the heart.

Isolated rat hearts were perfused according to the Langendorff method and preloaded with (^{3}H)-noradrenaline (20 μCi, specific activity 12.7 Ci/mmol) as previously described (Khan & Malik, 1980). Following the preloading, hearts were perfused with Kreb's solution for a further 60 min at the end of which 2 min heart perfusates were collected at 5 min intervals, 1 ml of each being counted in NE 260 Scintillator. The first perfusate was regarded as base-line value (100 %) and all subsequent values were expressed as a percent of this. In each study, control and ouabain-treated hearts were compared, and ECG was recorded throughout.

Ouabain (1×10^{-4}M) infused over a period of 15 min after collection of the second 2 min perfusate consistently produced arrhythmia. At 5, 10 and 15 min of ouabain infusion the % changes in tritium efflux were 68.2 ± 3.6, 67.2 ± 3.8 61.3 ± 4.9 % (means ± s.e. means). The corresponding values for control flux (in the absence of ouabain) were 90.5 ± 3.1, 81.0 ± 2.0 and 87.0 ± 3.2 %. The inhibition of efflux by ouabain was statistically significant ($P < 0.005$) and was reproducible at different times after (^{3}H)-noradrenaline infusion. Electrical stimulation of cardiac sympathetic nerve plexus at 3 Hz for 20 sec at 40 min intervals increased the efflux of tritium in the presence of ouabain to 111.6 ± 22.3, 107.3 ± 31.8 and 91.0 ± 26.9 % compared with 300.5 ± 17.0, 312.1 ± 21.2 and 257.6 ± 30.0 % in control hearts ($P < 0.005$). Infusion of ouabain did not significantly reduce tritium release induced by tyramine (1.5×10^{-6}M). Phenoxybenzamine (3×10^{-6}M) did not block the inhibitory effect of ouabain on the spontaneous efflux but did antagonise the inhibitory effect of ouabain on electrically-induced release of tritium. The inhibitory effect of ouabain on the spontaneous release was reduced but not abolished by the presence of either atropine (2.5×10^{-5}M) or indomethacin (1.4×10^{-5}M). Atropine antagonised the inhibitory effect of ouabain on electrically-induced release of tritium. Electrical stimulation in the presence of indomethacin increased the release by 301.7 ± 21.8 % but in the presence of indomethacin together with ouabain this was reduced to 203.8 ± 20.0 % ($P < 0.01$).

We conclude that ouabain in arrhythmogenic concentrations inhibits both spontaneous tritium release from the rat heart and that following electrical stimulation. The relationship between this inhibition and the inotropic and arrhythmogenic effects of ouabain is unclear and the role of calcium and presynaptic receptors in this inhibitory action remain to be investigated.

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URETHANE INCREASES PLASMA ADRENALINE LEVELS BY A CENTRAL MECHANISM THAT IS INDEPENDENT OF REFLEX PATHWAYS IN THE RAT

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Urethane-induced anaesthesia in rats is associated with an elevation in plasma concentrations of adrenaline (Piccotti et al., 1979). We have now investigated whether this effect is central and/or peripheral in origin.

Male rats (Sprague Dawley, 230-260 g) were anaesthetised with either urethane (1.2 g/kg, i.p.) or pentobarbitone (55-60 mg/kg, i.p.), bivagotomised, artificially ventilated and heparinised (1 mU/kg, i.v.). A group of animals were adrenalectomised (ADX) whilst anaesthetised with ether and 30 min later given either urethane or pentobarbitone. In some urethane-anaesthetised rats the lower arterial blood pressure was elevated by vasopressin (VASO) infusion (5.0 mU/kg/min at 0.08 ml/min) to levels measured in pentobarbitone-anaesthetised rats. In another group the carotid arteries were ligated to remove the influence of the barostatic reflex (CAROT). Finally, studies were made of the effects of urethane in rats previously pithed (PITH) during ether anaesthesia and in animals with the brain separated from the cord by transection immediately below it (BRX). Control animals were dosed with saline (0.4 ml/kg i.p.). In all preparations carotid arterial blood pressure (MAP) and heart rate (HR) were measured. A 1 ml blood sample was collected from the carotid artery for adrenaline (AD) and noradrenaline (NA) determination (Da Prada and Zürcher, 1976).

The table gives the plasma concentration of AD and NA (means, min and max) and MAP and HR ($\pm$ s.e. mean). Urethane anaesthesia was characterised by a high plasma level of AD. This effect was not prevented by elevating the low arterial pressure associated with this anaesthesia nor by ligating the carotid arteries. However, urethane failed to increase plasma AD in rats without central control.

GROUP	ANAESTHESIA	PROCEDURE	N	AD (pg/ml)	NA (pg/ml)	MAP (mm Hg)	HR (b/min)
1	PENTO	NONE	5	0	259 (186-460)	127 $\pm$ 3	438 $\pm$ 9
2		ADX	5	24 (0-64)	308 (149-452)	116 $\pm$ 6	428 $\pm$ 10
3	URETH	NONE	10	1437* (453-5365)	129 (15-261)	77 $\pm$ 4	428 $\pm$ 11
4		ADX	6	17 (0-43)	284 (103-674)	83 $\pm$ 7	427 $\pm$ 9
5		CAROT	5	2018* (911-3562)	226 (120-386)	76 $\pm$ 3	430 $\pm$ 12
6		VASO	6	961* (840-1184)	135 (92-185)	120 $\pm$ 4	426 $\pm$ 4
7		PITH	6	13 (1-30)	138 (106-170)	49 $\pm$ 1	309 $\pm$ 11
8		BRX	3	124 (53-228)	285 (237-271)	59 $\pm$ 4	307 $\pm$ 26

* $P<.05$ from groups 1, 2, 4, 7 and 8 (Mann-Whitney U test).

These findings suggest that urethane-induced elevation of plasma AD is mediated via an action on nervous structures above the cervical spinal cord and is not of reflex origin.

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AIRWAY RESPONSIVENESS TO SALBUTAMOL, AMINOPHYLLINE AND IPRATROPIUM Br AFTER CHRONIC SALBUTAMOL IN THE GUINEA-PIG

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Tolerance to $\beta_2$adrenoceptor agonist drugs has been reported in asthmatic subjects (Jenne et al, 1975). In this study guinea pigs were treated chronically with salbutamol to determine whether tolerance could be induced and whether tolerance extended to aminophylline and ipratropium bromide. Guinea pigs (male Duncan Hartley 450-550 g) were anaesthetized with i.p. α-choralose (50 mg/kg) and pentobarbitone Na (25 mg/kg). The animals were ventilated and lung resistance (R_L) computed from tracheal flow and transpulmonary pressure (Amdur and Mead, 1958). Histamine acid phosphate (3 μg/kg i.v.) was used as a standard bronchoconstrictor stimulus. Dose/response curves were produced using a control group of animals for each of the drugs, salbutamol, aminophylline and ipratropium bromide to attenuate the response to histamine. A further 3 groups were pretreated with salbutamol 15 μg/kg s.c. 3 times daily for 7 days and then investigated in the same way. For each of the 6 groups n = 6.

R_L (mmH_2O/ml/sec) at the start of the experiment was 2.31 ± 0.09(SEM) for control animals and 2.45 ± 0.10(SEM) for salbutamol-treated animals. The effects of salbutamol and aminophylline were significantly reduced by pretreatment. The ipratropium Br response was unchanged. Down regulation of $\beta_2$adrenoceptors by chronic treatment with salbutamol explains the reduced effect of this drug (Mukherjee et al, 1975). The reduced effectiveness of aminophylline indicates an action partially via $\beta_2$adrenoceptors, possibly by inducing catecholamine release (Atuck et al, 1967). The lack of effect of chronic salbutamol on the response to ipratropium bromide is not surprising. However it supports evidence that asthmatics who have a diminished response to $\beta_2$adrenoceptor agonists benefit from inhaled ipratropium bromide (Ward et al, 1981).

Changes in R_L (mmH_2O/ml/sec) in response to histamine(3 μg/kg) alone and after acute pretreatment (i.v.) with salbutamol or aminophylline or ipratropium bromide

		Histamine alone	Salbutamol (μg/kg)				Aminophylline (mg/kg)			Ipratropium bromide (μg/kg)			
			1	2	5	10	5	10	20	1	2	5	10
Untreated controls	Mean	2.38	1.57	1.12	0.45	0.12	1.72	1.27	0.65	1.57	1.36	0.67	0.67
	SEM	0.21	0.28	0.23	0.11	0.07	0.57	0.37	0.20	0.15	0.19	0.09	0.10
				*	*	***		*	**				
Chronic Salbutamol	Mean	2.91	1.88	2.03	1.62	1.10	3.32	3.38	2.12	2.08	1.90	1.03	0.93
	SEM	0.26	0.24	0.23	0.37	0.17	0.86	0.85	0.43	0.30	0.35	0.21	0.23

*p < 0.05; **P < 0.01; ***p<0.001 by unpaired t-test.

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A COMPARISON OF PULMONARY REACTIVITY TO INHALED ANTIGENS OR HAPTENS IN SENSITIZED GUINEA-PIGS

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The non-invasive forced-oscillation method of Swann et al.(1967) and Hiett, (1974), for evaluation of respiratory function, was applied to characterise the nature of hypersensitive reactions provoked by inhalation of a variety of sensitisizing agents. This method, using a combination of plethysmographic pneumotachographic, and pulmonary resonance measurements, provided values for respiration rate, tidal volume, airway resistance and compliance in conscious guinea-pigs, allowing continuous or repeated observations to be made. The methodology was evaluated by measurements of anaphylactic reactions compared with lesions of Type-III or Type-IV character, all provoked by inhalation of protein antigens in previously sensitized animals. It was then attempted to detect similar changes resulting from comparable sensitizing and challenging procedures with chemical haptens.

Anaphylactic reactivity was evaluated in guinea-pigs sensitized (Matsumura, 1970) by exposures for I hour to inhalation of an aerosol of ovalbumen (0.75 % w/v solution), repeated 7 days later. On day 15, the animals were exposed further to aerosols of 0.01 %, 0.1 % and I% solutions successively at 15 minute intervals. Pulmonary function was assessed at I minute intervals. Exposures to the 0.1% and I% concentrations gave significant elevations of rate and airway resistance, decreased tidal volumes and markedly decreased compliance measurements. No changes were observed in unsensitized controls.

In further experiments the animals were sensitized by subcutaneous or foot-pad injections of antigen or hapten in Freund's complete adjuvant (FCA) or FCA alone, given on days I and I5. The animals were challenged on day 29 by I hour exposures to inhalation of antigen or hapten aerosols and functions were measured immediately after exposure and 3 hours, 24 and 48 hours later.

One group was sensitized with ovalbumen 0.25mg. in FCA and challenged with 0.5 % ovalbumen aerosol. After 3 hours respiration rate had increased and tidal volume diminished. Airway resistance rose slowly over 3 hours. There was severe respiratory difficulty. Roska et al.(1970) described haemorrhagic pneumonitis in similar animals and indicated a Type-III mechanism. In guinea-pigs sensitized with FCA alone and challenged with PPD (10,000 u/ml.) aerosol, an increased rate and decreased tidal volume were observed after 24 and 48 hours but not at I or 3 hours. There were no differences in airway resistance. Because of large variation in both experiments, possible changes in compliance were obscured. It has been indicated that such PPD-induced reactions are due to Type-IV hypersensitivity (Miyamoto et al.1971).

In animals sensitized using either potassium dichromate or dinitrochloro--benzene (DNCB), both Img., in FCA, and challenged respectively with aerosols of 0.01% or I% solutions, no effects attributable to sensitization with the haptens could be shown.

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CHARACTERIZATION OF CYCLIC NUCLEOTIDE PHOSPHODIESTERASES IN GUINEA-PIG LUNGS

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The intracellular chemical messengers cyclic AMP and cyclic GMP have been implicated in bronchial asthma. The turnover of cyclic nucleotide is controlled by nucleotide cyclases and by cyclic nucleotide phosphodiesterases (CN PDE). Although the inhibitors of CN PDE, theophylline and aminophylline, are of proven value in the treatment of asthma, there is still considerable uncertainty as to whether their action on bronchial muscle tone and mast cell degranulation is produced through inhibition of CN PDE.

Initial experiments were directed towards examining the properties of CN PDE in guinea-pig lung tissue. Activity of cyclic AMP and cyclic GMP PDE was determined by measuring the rate of decrease of ^{3}H labelled substrates. Unhydrolysed labelled substrate was separated from hydrolysed products by neutral alumina chromatography; recovery from each alumina column was monitored with ^{14}C labelled substrate (Methven et al 1980). Kinetic studies revealed the presence of multiple forms of CN PDE in guinea-pig tissue. Time-course and protein dependence studies were performed at three substrate concentrations (1000, 20 and **5** µM of each cyclic nucleotide) using the 10^{5} g supernatant enzymes. The apparant Km values calculated by linear regression analysis indicated low and high affinity supernatant enzymes for cyclic AMP (see Hitchcock, 1973) and for cyclic GMP. The subcellular distribution of high affinity CN PDE was determined in sequential particulate fractions separated at $1.6^{3}g$, $12^{3}g$ and $10^{5}g$, and the $10^{5}g$ supernatant at a substrate concentration of 5 µM. Unlike the findings of Davis and Kuo (1977) the high affinity cyclic AMP was about equally distributed between the total particulate fractions (53%) and $10^{5}g$ supernatant (47%), cyclic GMP PDE was mainly particulate (61%, $p<0.02$). The detergent, Triton X-100 (1% w/v) strongly inhibited both the high affinity cyclic AMP and cyclic GMP, $1.6^{3}g$ particulate and $10^{5}g$ supernatant PDE enzymes. In preliminary experiments we have examined the inhibitory action of theophylline and aminophylline ($10^{-2}M$ to $10^{-6}M$) on the high affinity, particulate and $10^{5}g$ supernatant enzymes. So far, complex inhibitory profiles have been observed with theophylline and aminophylline. Experiments are in progress to determine the I_{50} values for particulate and supernatant, high and low affinity cyclic AMP and cyclic GMP phosphodiesterases.

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ANTIGEN-INDUCED BRONCHIAL ANAPHYLAXIS IN SD RATS: POSSIBLE MEDIATORS INVOLVED

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The effects of various pharmacological antagonists on anaphylactic bronchoconstriction in immunized and anaesthetized rats were studied. Sprague Dawley (SD) rats were immunized i.p. with 100 µg ovalbumin (OA) together with 100 mg $Al(OH)_3$. The animals were tested two to six weeks after immunization and challenged i.v. with a low (0.5 mg OA, ΔP %) and a high (5 mg OA, ΔPmax %) antigen dose. The respiratory measurements were made essentially as described by Stotland and Share (1974), Dahlbäck (1981). The drugs were given by i.v. administration two or five minutes before OA provocation. Table 1 shows the effect of various drugs when used alone or in combination.

Table 1 Effects of pharmacological antagonists on anaphylactic bronchoconstriction in rat

Drug	Dose mg/kg, i.v.	n	% inhibition of bronchial anaphylactic response Δ P%	Δ Pmax%
Methysergide	0.05	31	64 ± 5***	48 ± 5***
1-Benzyl imidazole	10	15	64 ± 7***	48 ± 7***
BW 755C	1	10	66 ± 10***	+1 ± 5
Indomethacin	5	24	46 ± 11***	36 ± 4***
Atropine	1	3	16 ± 40	5 ± 14
Methysergide + Indomethacin	0.05 + 5	10	98 ± 1***	91 ± 2***
Methysergide + 1-Benzyl imidazole	0.05 + 10	10	92 ± 4***	86 ± 2***
Methysergide + BW 755C	0.05 + 1	10	70 ± 6**	45 ± 9***
Indomethacin + 1-Benzyl imidazole	5 + 10	5	13 ± 29	51 ± 6**

** $p < 0.01$ *** $p < 0.001$

Bilateral vagotomy did not change the anaphylactic response. Possible mediators were also examined for their capacity to induce changes of the intratracheal pressure after i.v. administration. The rank order of potency was acetylcholine $\geq$ 5-HT $\geq$ bradykinin > PGF_2 >> histamine = PGE_1 = PGE_2 = 0.

The results show that methysergide inhibits the major part in the rat lung anaphylaxis, whereas the other part of the antigen contraction is inhibited by 1-benzyl imidazole (a thromboxane synthetase inhibitor (Flower 1981)) and by indomethacin. A combination of these two does not show any additive inhibition. BW 755C could only inhibit at the low provocation and showed no additive protection in combination with methysergide. It is noticeable that BW 755C did not inhibit at the high provocation dose but further investigations must be done to find an explanation. In the present study, acetylcholine, another constrictor of the rat lung, was judged not to be a mediator on deeply anaesthetized animals devoid of vagal lung control.

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UNCHANGED RESPONSIVENESS TO BRONCHODILATORS IN TRACHEAS OBTAINED FROM RATS TREATED WITH ENPROFYLLINE FOR SIX MONTHS

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Exposure of bronchial smooth muscle to high concentrations of bronchodilators for prolonged periods of time may induce tolerance. This phenomenon is shown with β-adrenoceptor stimulants studied in vitro and in vivo. Also theophylline, reportedly may decrease the sensitivity to itself in airway smooth muscle (Benoy et al. 1975). Enprofylline (3-propylxanthine) is about 5 times as potent as theophylline as a bronchodilator in animals and man (cf Persson & Kjellin 1981). However, enprofylline does not produce theophylline-like diuresis and CNS-stimulation, supposedly reflecting a low ability of enprofylline to antagonize endogenous adenosine (Persson et al 1981). We have now examined the effects of enprofylline and isoprenaline in vitro in tracheas obtained from rats that have been treated with oral doses of enprofylline for 6 months.

Eighty-six rats were kept under highly standardized conditions with free access to either placebo food pellets or pellets containing enprofylline, corresponding to a daily intake of 100 μmol/kg, 200 μmol/kg or 600 μmol/kg. (The study was performed at the Toxicological Laboratories, AB Astra.) After 6 months the rats were sacrificed and a ring from the distal portion of the trachea was dissected out, cut open and mounted in organ baths containing heated (37° C), carbogenized Krebs solution (cf Karlsson & Persson 1981). Isometric tension changes were recorded. Carbacholine-contracted rings (0.44 μM corresponding to the EC50 of carbacholine) were relaxed by cumulative additions of enprofylline or isoprenaline. Relaxant pD_2 values to enprofylline and isoprenaline obtained in tracheas from any group of enprofylline-treated rats were not different from those obtained in untreated animals (Table 1). The prolonged pretreatment with enprofylline further did not change the sensitivity to carbacholine (which contracted all preparations to 0.93 ± 0.03 g, mean ± SEM). Nor did it affect the maximum relaxations induced by enprofylline (0.54 ± 0.04 g at 2.9 mM) or isoprenaline (0.36 ± 0.03 g at 3.6 μM). In agreement with earlier studies (Karlsson & Persson 1981) this latter result indicates that xanthines produce larger relaxations than β-receptor agonists.

Table 1

Daily dose of enprofylline for 6 months		Enprofylline, in vitro pD_2-values (Mean ± SEM)				Isoprenaline, in vitro pD_2-values (Mean ± SEM)			
	n	male	n	female	n	male	n	female	n
0	12	3.97 ± 0.07	7	3.85 ± 0.04	5	7.24 ± 0.10	7	7.36 ± 0.08	5
100 μmol/kg	10	3.99 ± 0.13	5	3.92 ± 0.09	5	7.27 ± 0.17	4	7.22 ± 0.06	5
200 -"-	11	3.93 ± 0.08	6	3.87 ± 0.08	5	7.31 ± 0.10	5	7.23 ± 0.02	5
600 -"-	11	3.93 ± 0.09	5	3.87 ± 0.05	6	7.39 ± 0.06	5	7.36 ± 0.07	6

Six months is a considerable portion of the life span of the rat. It is also a much longer period of treatment than hitherto used in studies on the development of tachyphylaxis to bronchodilators in animals. Furthermore, the daily doses of enprofylline used were much above the calculated daily dose, about 50 μmol/kg, in man. In both rats and man enprofylline is almost completely absorbed after oral intake and excreted as unchanged drug in the urine. In conclusion, long-term enprofylline treatment did not affect the in vitro tracheal responsiveness to itself or to isoprenaline.

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ENHANCEMENT BY BETAMETHASONE OF THE EFFECTS OF BRONCHODILATOR DRUGS ON METHACHOLINE-INDUCED BRONCHOCONSTRICTION IN RATS

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Corticosteroids are known to enhance the effects of β-adrenergic stimulation in lungs (Middleton 1975; Holgate et al, 1977). The present study was conducted in order to evaluate the possible interaction of a glucocorticoid, betamethasone (BM), with three bronchodilator drugs of different mode of action on the airways: terbutaline (TER), theophylline (THEO), and ipratropium bromide (IPRA).

Female Sprague Dawley rats weighing 200-250 g were anaesthetized with pentobarbitone, relaxed with pancuronium, and artificially ventilated. Peak intratracheal pressure responses (PIPR), blood pressure (BP), and heart rate (HR) were recorded as previously described (Salonen & Mattila 1981). The rats were injected i.v. at 2-min intervals with consecutive doses of methacholine (MeCh)(2, 3, and 4.5 μg), and the respective responses were measured. Pretreatment with BM (0.4 mg/kg i.p.) was given 24 h before the experiment, other rats receiving 0.5 ml of saline, respectively. Acute doses of BM or saline were given 12 min and those of TER, THEO, or IPRA 5 min prior to starting the MeCh challenge.

Table 1 Modification by bronchodilator pretreatment of the bronchial and cardiovascular effects of MeCh (2, 3, and 4.5 μg). Statistical significances (Duncan's test; a=p<0.05 and b=p<0.01) refer to differences from the controls. Cardiovascular effects refer to MeCh 4.5 μg.

Treatment	PIPR (mmHg)			Cardiovascular	
	2 μg	3 μg	4.5 μg	BP(mmHg)	HR(min^{-1})
Controls	2.6±0.3	$5.7±0.6_a$	10.4±0.9	34±4	347±15
BM 0.4 mg/kg	$1.3±0.5^b$	$3.6±1.0^a$	7.8±1.5	45±7	387±17
TER 20 μg/kg	$0.8±0.2^b$	$2.7±0.9^b$	$6.5±1.7^a$	44±8	350±28
BM 0.4+TER 20	$0.3±0.2^b$	$1.0±0.3^b$	$3.0±0.9^b$	43±8	377±19
THEO 20 mg/kg	$1.2±0.3^b$	$2.8±0.4^b$	$5.5±1.2^b$	33±4	$367±40_b$
BM 0.4+THEO 20	$0.6±0.1^b$	$1.6±0.3^b$	$2.9±0.6^b$	55±6	$470±15^b$
IPRA 0.5 μg/kg	$1.0±0.3^b$	$2.6±0.6^b$	$4.6±0.7^b$	$44±6_b$	$403±8_b$
BM 0.4+IPRA 0.5	$0.8±0.2^b$	$1.3±0.3^b$	$2.3±0.2^b$	$80±9^b$	$453±16^b$

As seen in Table 1 (mean±s.e.mean values) BM 0.4 mg/kg but not BM 2 mg/kg exerted moderate bronchodilation and enhanced the effects of all three bronchodilators, best that of TER. The combined effect of BM+TER was seen only on airway responses, while BM+THEO and BM+IPRA also counteracted effects of MeCh on circulation. A time interval of 24 h after BM pretreatment was necessary for the action of BM. This tallies with the findings of Church (1975) and supports the concept of interference between corticosteroids and β-adrenergic stimulation at the level of adenylate cyclase or phosphodiesterase synthesis (Middleton 1975).

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A NONCHOLINERGIC NONADRENERGIC NEURONAL COMPONENT MEDIATING SLOWLY DECLINING CONTRACTION IN THE GUINEA-PIG AIRWAYS

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The innervation of the guinea-pig airways shows a growing complexity. The existence of three neuronal components in the tracheal muscle seems well established (Coburn & Tomita, 1973; Grundström et al, 1981). These components are: the cholinergic, the noradrenergic and the noncholinergic nonadrenergic inhibitory component (possibly VIP according to Matsuzaki et al, 1981). Recently we made a more extensive investigation on the innervation of the guinea-pig airways (Grundström et al, 1981), where we also included the main bronchi and the hilus bronchi as shown in Figure 1. In the main bronchi we could only observe a cholinergic contractile component and in the hilus bronchi we reported of a new noncholinergic nonadrenergic neuronal component of contraction (NNCC), which was the sole mediator of contraction.

In our investigations we have used field stimulated ring preparations as shown in Figure 1. Isometric tension was recorded in the absence and presence of various pharmacological agents. Stimulation was performed as trains of 1 ms squarewave pulses with varying duration and frequency of the pulse trains. In the present study we have been able to observe NNCC:s also in the main bronchi and occasionally in the trachea i.e. stimulation with pulse trains of 5 Hz and 30 s duration repeated every 10 min elicited contractions that were resistant to atropine (1.1 μM), guanethidine (10 μM) and propranolol (3.4 μM) and were completely abolished by TTX (1 μM). The decline following the stimulated contraction is slow (half time around 5 min) similar to the NNCC previously observed in the hilus bronchi.

We suggest that there exists a NNCC of similar character in those parts of the guinea-pig airways studied so far. The NNCC is mainly located to the hilus and main bronchi but may occasionally appear in the trachea. The contraction that is mediated by this NNCC declines in a slow manner which makes it interesting in relation to sustained contractions and muscle tone in the airways. Could the existence of NNCC:s also be established in human airways it would accordingly have implications for the mechanisms of asthma.

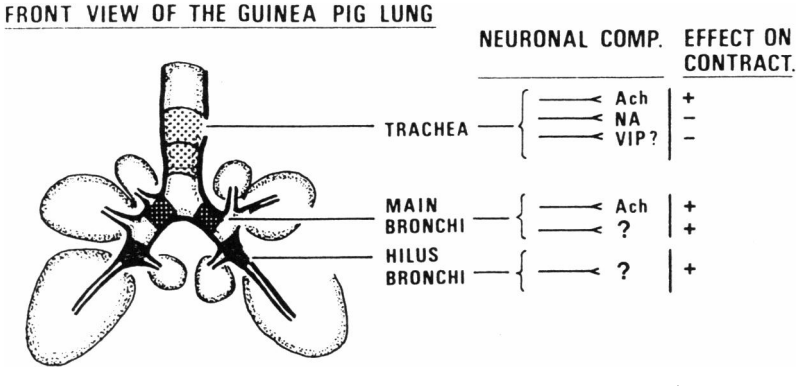

Figure 1 View of the guinea-pig lung showing the position of the preparations used and the possible innervation of the related smooth muscles.

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EFFECTS OF ADRENALINE AND TERBUTALINE ON MEDIATOR-INCREASED VASCULAR PERMEABILITY IN THE CAT TRACHEA

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We have previously shown that terbutaline (given s.c.) was about equipotent to adrenaline to inhibit the development of pulmonary edema in guinea-pigs exposed to a histamine aerosol (Persson and Erjefält, 1979). To study more specifically effects in the airways a method utilising cat trachea was developed. In barbiturate-anaesthetized cats, previously given Evans blue dye 30 mg/kg i.v., the trachea was opened ventrally about 4 cm by a longitudinal incision. Three or four randomised 50 µl injections of saline, histamine, substance P or bradykinin were made into tracheal tissue away from the incision line. A local blueing occurred immediately after mediator deposition in the trachea (similar injections of histamine made subpleurally were followed by involution of the injected area (cf Persson and Ekman 1976), but blueing was not seen). Histamine was also given together with adrenoceptor agonists and antagonists. After 15 min dye was extracted from each tracheal injection area and determined by absorbance readings. These values were used as a measure of vascular efflux of macromolecules. Substance P and bradykinin significantly increased the dye exudation but were less potent than histamine. The results with histamine and other drugs are summarized in Table 1.

Table 1 Evans blue dye leakage, cat trachea

Injection 50 µl	Absorbance (Mean ± SEM)	n
Saline	0.315 ± 0.022***	20
Histamine 0.1 µg (=His)	0.634 ± 0.041	25
His + Propranolol 0.2 µg (=Propr)	0.644 ± 0.034	7
His + Terbutaline 0.1 µg (=Terb)	0.477 ± 0.073*	8
His + Propr + Terb	0.606 ± 0.023	10
His + Adrenaline 0.1 µg (=Adr)	0.239 ± 0.028***	8
His + Adr + Propr	0.241 ± 0.051**	4
His + Adr + Phentolamine 1 µg	0.413	2

* $p<0.02$; ** $p<0.01$; ***$p<0.001$. Students t-test on differences from histamine control (0.634)

It may be concluded that: (1) Locally injected mediators increased microvascular permeability to macromolecules in tracheal tissue. (2) Terbutaline, by stimulating β-receptors, reduced the mediator- (histamine-) induced edema, in agreement with the notion that a β-receptor function at the microvascular membrane inhibits permeability (Svensjö et al 1976, Persson et al 1982). (3) Adrenaline, probably via stimulation of flow regulating α-receptors, completely inhibited the histamine--induced edema and produced a "blanching" effect. - It is not known to what extent a "blanching" effect would be a desirable drug effect in the lower respiratory tract. On the other hand, the existence of a vascular permeability reducing β-receptor function in the airways offers an additional aspect on the value of β-2-receptor stimulants, such as terbutaline, as profylactic asthma drugs.

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BUFURALOL: A β-ADRENOCEPTOR ANTAGONIST WITH BRONCHODILATING PROPERTIES

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Bufuralol (1-(7-ethylbenzofuran-2-yl)-2-tert-butylamino-1-hydroxyethane) is a non-selective β-adrenoceptor antagonist with partial agonist activity (Fothergill et al; 1975). The agonist activity exerted by this compound and its metabolites has been shown to be, to a large extent, at the β_2-adrenoceptor (Hamilton and Chapman; 1978). The effects of ±-bufuralol have been compared to those of ±-propranolol in the anaesthetised cat.

Cats (2.5 - 3.0 kg) were anaesthetised with α-chloralose (60 $mg.kg^{-1}$ i.p.) and sodium pentobarbitone (6 $mg.kg^{-1}$ i.p.) and the trachea cannulated. Blood pressure was recorded from the left carotid artery and the right external jugular vein was catheterised for drug administration. The right femoral vein was also catheterised for the infusion of drugs.

Airways resistance (R_{aw}) and dynamic lung compliance (C_{dyn}) were measured by the subtractor method as described by MacLagan and Ney (1979). Airflow and tidal volume were measured through a Fleisch flow transducer (size 00) connected to a Gould-Godart Pneumotachograph. Trans-pulmonary pressure was recorded by means of a Mercury (M10) micromanometer which measured the pressure difference between the tracheal cannula and a trocar (13g) inserted into the intrapleural cavity. The control values (N=5) for R_{aw} and C_{dyn} were 15.3 ± 3.8 cm $H_2O.l^{-1}.s^{-1}$ and 10.6 ± 1.8 ml.cm H_2O^{-1} respectively. Administration of ± bufuralol or ±-propranolol in doses of 1 μg - 1 $mg.kg^{-1}$ i.v. did not change R_{aw} or C_{dyn}. Infusion of 5-hydroxytryptamine HCl at a rate of 10 $\mu g.kg^{-1}.min^{-1}$ increased R_{aw} to 38.0 ± 2.6 cm $H_2O.l^{-1}.s^{-1}$ and decreased C_{dyn} to 6.0 ± 0.8 ml.cm H_2O.

In the presence of 5-hydroxytryptamine ±-bufuralol produced a decrease in R_{aw} and small increase in C_{dyn} (approximately 8%) which were not dose related; ±-propranolol increased R_{aw} and decreased C_{dyn} in a dose related manner (Table 1).

Table 1 Percentage change in airways resistance

Dose (kg^{-1})	10 μg	30 μg	100 μg	300 μg	1 mg
±-bufuralol	-34.5 ± 4.7	-30.4 ± 2.4	-30.6 ± 1.9	-22.2 ± 7.0	-38.1 ± 4.4
±-propranolol	+20.8 ± 0.2	+22.8 ± 0.5	+28.1 ± 3.1	+45.8 ± 9.3	+96.2 ± 2.1

(N=3)

These results show that ± bufuralol produces bronchodilation in the anaesthetised cat when bronchoconstriction has been previously induced whereas ±-propranolol produces further bronchoconstriction.

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α AND β-ADRENOCEPTORS IN THE TRACHEOBRONCHIAL TREE OF THE RABBIT

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Electrical stimulation of sympathetic nerves and phenylephine produced contraction of the trachealis muscle in the rabbit *in vivo*. Both responces were blocked by phenotalamine. This indicates the presence of α-adrenoceptors in the rabbit trachea (Mustafa et al, 1982). Further experiments were carried out to study the regional distribution of α - & β-adrenoceptors in the tracheobronchial tree of the rabbit.

In vivo studies (n = 8) showed that adrenaline (1 μg/kg i.v.) caused relaxation of rabbit trachea previously contracted by carbachol (2 μg/kg i.v.) while in the presence of propranolol (1 mg/kg i.v.), adrenaline produced a contraction which was blocked by the α_1-adrenoceptor antagonist prazosin (1 mg/kg i.v.).

In vitro (n = 10) adrenaline (6×10^{-6} - 5×10^{-4}) caused relaxation of ring preparations obtained from both trachea and small bronchi. This effect was blocked by propranolol (3×10^{-6}M). After propranolol, adrenaline (6×10^{-6} - 5×10^{-4}) caused contraction of the trachea and had no effect on the small bronchi. Noradrenaline (1.4×10^{6} - 2×10^{-4}M) in the presence or absence of propranolol produced contraction of the trachea but had no effect on small bronchi. These responses were blocked by prazosin (3×10^{-6}). The results are summarized in Table I.

Table 1

IN VIVO : TRACHEAL MUSCLE PREPARATION

Carbachol	Adrenaline	Adrenaline+ Propranolol	AD+ Prazosin+ Propranolol
Carbachol Contraction	Relaxation*	Contraction*	No effect*

* Effect tested on tracheal muscle previously contracted by carbachol 2.0 μg/kg

IN VITRO : RESPONSES CALCULATED AS % OF THE MAXIMUM RESPONSE PRODUCED BY CARBACHOL

Preparation	Carbachol	NA	NA Propranolol	NA Prazosin	AD	AD+ Propranolol
Trachea	Cont. 100%	Cont. 33%	Cont. 32%	Blocked 18%	Relax. -35%	No effect
Small Bronchi	Cont. 100%	No effect	No effect	-	Relax. -13%	No effect

NA = Noradrenaline, AD = Adrenaline

We therefore, conclude that β-adrenoceptors are present in both trachea and small bronchi of the rabbit, whereas α_1-adrenoceptor activity was only found in the trachea.

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DOES THE (+)-ISOMER OF TERBUTALINE BLOCK β-ADRENOCEPTORS?

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The agonistic activity of racemic β-adrenoceptor agonists resides almost exclusively in the (-)-isomer and this is the case also for terbutaline (TRB) (Wetterlin, 1972). It has been proposed, however, that the nonagonist isomer of a β-adrenoceptor agonist might possess a blocking action thus resulting in partial agonist activity of the racemate (Bowman, 1980). We have investigated this possibility and have studied the β-adrenoceptor mediated effects of (-)- and (+)-TRB on tissues from the guinea-pig. The trachea (a tissue containing both β_1 and β_2 adrenoceptors), the soleus muscle (β_2) and the papillary muscle from the left ventricle (β_1) were mounted in organ baths containing oxygenated Krebs solution at 37°C and were prepared for isometric recording.

(+)-TRB was about 3,000 times less potent than (-)-TRB in relaxing the carbachol-contracted (0.05 μM) trachea and was even less potent in depressing subtetanic contractions of the soleus muscle (table 1). The presence of 5 μM (100 times the EC_{50} for (-)-TRB) (+)-TRB did not inhibit the effects of (-)-TRB on these tissues. Propranolol, 0.1 μM, blocked the effect of (+)-TRB on the trachea but to a lesser extent than it blocked (-)-TRB ($P<0.005$) indicating that part of the relaxing effect of (+)-TRB on this organ was unspecific. This may explain why the potency ratio of the isomers appeared higher for the soleus than for the trachea.

The inotropic effect of (-)-TRB on the papillary muscle was about 200 times weaker than the effect on the soleus ($P<0.001$) thus confirming its β_2-selectivity. Moreover, (-)-TRB was a partial agonist on the papillary muscle when compared with (-)-isoprenaline ($\alpha=0.74\pm0.02$, n=8). (+)-TRB had a negligible effect per se, nor did it inhibit the effect of isoprenaline: The pD_2 for (-)-isoprenaline on the papillary muscle was 8.64 ± 0.10 (8) and 8.62 ± 0.17 (5) in the presence and in the absence, respectively, of 100 μM (+)-TRB.

Table 1 β-adrenoceptor mediated effects of the enantiomers of terbutaline

	mean $pD_2 \pm$ s.e.m. (n)		
Tissue Effect	Trachea relaxation	Soleus muscle depr. of contr.	Papillary muscle inotropy
(-)-TRB	7.35 ± 0.03 (6)	7.36 ± 0.06 (5)	5.03 ± 0.08 (8)
(+)-TRB	3.84 ± 0.11 (3)	≤ 2.7** (3)	Nil
(-)-TRB*	7.38 ± 0.03 (3)	7.51 ± 0.01 (3)	-

*In the presence of 5 μM (+)-TRB
**At 2 mM the effect of (+)-TRB was ≤ 50 % of the max. eff. of (-)-TRB

In conclusion, our results show that (+)-TRB has virtually no affinity for β-adrenoceptors and is devoid of any blocking activity. Also, the (+)-isomer of prenalterol, a partial β_1-adrenoceptor agonist has a very weak affinity for β-adrenoceptors as compared with its (-)-isomer (Johansson & Waldeck, 1980). Thus the dualistic action of a partial β-adrenoceptor agonist in racemic form seems to reside in one isomer only.

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SPONTANEOUS AND EVOKED ACTIVITY AT A SYMPATHETIC NEURO-EFFECTOR JUNCTION

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Useful information has been obtained by comparing spontaneous and evoked activity at the skeletal neuromuscular junction. Similar comparisons have been less useful at sympathetic neuro-effector junctions as more than one release site contributes to the excitatory junction potential (e.j.p.).

Peaks may be revealed in the rate of depolarization of the e.j.p. in the rodent vas deferens by differentiation with respect to time. They have been called Discrete Events (DE's) (Blakeley & Cunnane (1979)). DE's occur at fixed latencies, are intermittent, and vary in amplitude in a stepwise manner suggesting that they, like spontaneous events, reflect transmitter action at a single site. Within a given latency band, variation in DE amplitude may be due to the release of a variable number of 'packets' of transmitter from one release site, or the release of single packets of transmitter at different positions relative to the recording electrode (perhaps different varicosities on the same nerve).

We present the results of a theoretical consideration of current spread in smooth muscle, based on models of Bennett (1972) and Purves (1976). We suggest that the major variation in DE amplitude is not likely to be due to the release of single packets of transmitter at sites separated from the recording electrode by sufficiently different distances to affect the amplitude of the recorded response.

If several sites contribute DE's of different amplitudes at the same latency, then the amplitudes of DE's and the differential of spontaneous events should vary in the same way. If single sites contribute to variable number of quanta, then the differential of spontaneous events should correspond with the smallest classes of DE's.

Spontaneous junction potentials (s.e.j.p's) are observed with amplitudes from noise level to many millivolts. The largest are least frequent. Small s.e.j.p's have rates of rise indiscriminable from the noise of our differentiator. Only the largest s.e.j.p's yield differentiated signals comparable with discrete events.

The smallest s.e.j.p's have a slower time course, and we attribute them to release sites remote from the recording electrode. The amplitudes of the largest differentiated s.e.j.p's are comparable to the smallest classes of evoked DE's.

It remains most likely, therefore, that DE's reflect the release of a variable number of packets of transmitter from either a single release site or a small group of closely associated release sites on the same cell close to the recording electrode.

The MRC are thanked for their support.

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CHOLINERGIC SUPPRESSION OF ADRENERGIC NEUROTRANSMISSION IN CANINE CORPUS CAVERNOSUM URETHRAE (CCU)

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Evidence has been presented indicating that in the isolated field stimulated retractor penis muscle (rp) of dog and several other species motor adrenergic neurotransmission is suppressed by cholinergic nerves (Klinge & Sjöstrand 1977a). Such evidence has not been presented for the smooth muscles in the penile erectile bodies. In this study strips of dog ccu were mounted in Tyrode solution and stimulated transmurally (0.5 ms, 5-10 Hz, 10-35 V, 10-20 s trains). Addition of only scopolamine (S)(0.0026-0.52 μM) to the organ bath enhanced the adrenergic excitatory responses. Physostigmine (P) by itself diminished these responses (Figure 1). Subsequent addition of S not only promptly abolished the effect of P but rendered the contractions bigger than they were before application of P. Also acetylcholine (ACh)(50-300 μM) diminished the contractions and its effect was augmented by P and abolished by S.

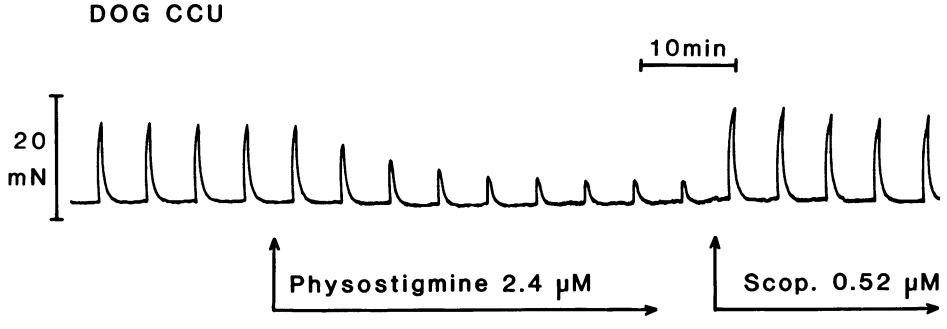

Figure 1 Effect of physostigmine and scopolamine on the contractions induced in canine ccu by activation of adrenergic nerves by field stimulation.

The ChE activity and the ACh content of the dog ccu are unknown but both are high in the dog rp (Bell & McLean 1970; Klinge & Sjöstrand 1977a). In a single species the innervation of the rp and the ccu seems to be similar (e.g. Eränkö et al 1976; Klinge & Sjöstrand 1977a, b). In the dog ccu stimulation of muscarinic receptors located in the smooth muscle cells does not cause relaxation (Klinge & Sjöstrand 1977b). Consequently, the effects of P and S revealed that the contractions could be diminished by an intrinsic muscarinercic mechanism probably operating by suppression of motor adrenergic neurotransmission according to the theory of Muscholl (1979).

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CO-RELEASE OF VIP AND ACETYLCHOLINE IN RELATION TO AUTONOMIC CONTROL OF VASODILATION AND EXOCRINE SECRETION

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Recent evidence suggests that VIP and acetylcholine (ACh) coexist in postganglionic neurons around blood vessels and acini of cat exocrine glands (see Lundberg, 1981'). Simultaneous release of VIP and ACh in vivo during nerve stimulation was demonstrated in the venous effluent from the cat submandibular salivary gland using radioimmunoassay (VIP) and gaschromatography and masspectrometry (ACh). Local eserine infusion was a prerequisite for detection of ACh. Eserine markedly potentiated the salivary secretion and vasodilation both at high and low frequencies during pre- or postganglionic nerve stimulation. The potentiating effect of eserine was abolished by atropine. Nerve stimulation caused a frequency-dependent ACh release which was maximal after 15 s of stimulation and then gradually declined. The ACh output per nerve impulse (about 200 fmol) was rather similar both at 2 and 10 Hz. After atropine ACh output increased about 2-fold during nerve stimulation. VIP release could easily be demonstrated in the non-eserinized animal suggesting a less efficient inactivation. VIP output gradually increased during the first five min of the stimulation period. The VIP output per nerve impulse was considerably higher at 10 Hz (1 fmol) than at 2 Hz (0.2 fmol). Atropine increased VIP output about 5-10-fold at 10 Hz, while no change was seen at 2 Hz. After a longterm stimulation (10 Hz for 1 h) the tissue levels of VIP in the submandibular gland were markedly lower than those on the non-stimulated side. The ACh content of the gland did not change after the stimulation indicating a local synthesis. The vast majority of VIP nerves in the submandibular gland occurred around exocrine elements, while the VIP nerves in the tongue were almost exclusively associated with blood vessels. Chorda-lingual nerve stimulation caused a marked vasodilation in both the tongue and the submandibular gland. At 2 Hz the vasodilation was significantly reduced in both organs by atropine, while at 15 Hz the vasodilation still was somewhat reduced in the tongue, while the submandibular vasodilation was atropine-resistant and prolonged compared to control stimulations. In the submandibular gland the VIP output was markedly increased after atropine at 15 Hz, while it did not increase in the tongue. This suggests that VIP release from nerves around exocrine elements but not the release from vascular nerves increases after atropine. The prolonged vasodilation in the submandibular gland after atropine may therefore be due to increased VIP release from nerves around exocrine glands which activates vascular receptors. No evidence so far suggests that secretory activity influences VIP degradation. No potentiation of the vasodilatory response was seen during combined infusions of VIP and ACh. The salivary response to ACh was however potentiated by addition of VIP. This was observed both with regards to the salivary volyme, protein and electrolyte (Na^+, Cl^-, K^+ and HCO_3^-) output. ACh by itself induced very high Na^+ and Cl^--levels in saliva compared to that seen at nerve stimulation. In conclusion, the nervous control of the vasodilatory and the salivary responses differs in several aspects. Vasodilation is atropine--sensitive at low frequencies and atropine-resistant at high frequencies indicating a frequency-dependent release of ACh and VIP. This is also supported by the observed characteristics of ACh and VIP release. The secretory response is totally abolished by atropine but in contrast to the vascular nerves, VIP release from nerves around exocrine elements seems to increase after atropine (suggesting a presynaptic modulation of release by ACh). Furthermore, VIP potentiates the secretory response to ACh.

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SIMULTANEOUS PURIFICATION OF CATECHOLAMINE SYNTHESIZING ENZYMES FOR THE RADIOENZYMATIC PHENYLETHYLAMINE ASSAYS

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The sensitive and specific radioenzymatic assays for phenylethylamine,tyramine, octopamine and phenylethanolamine need prior purification of dopamine beta-hydroxylase (DBH) and phenylethanolamine N-methyl transferase (PNMT). With a modification of the original procedures both enzymes could be purified from the same bovine adrenal medullae (Axelrod, 1961; Rush et al, 1974).

Fresh bovine adrenal glands were obtained from a local slaughterhouse. The glands were removed and placed in crushed ice 15-30 minutes after the exsanguination of the animals. All purification procedures were carried out at 4 ^{o}C. The cortex was removed as soon as possible, and the medullae were homogenized in four volumes of 0.32 M sucrose with a Potter-Elvehjem homogenizer fitted with a Teflon pestle (clearence 0.08 mm). The homogenate was centrifuged at 6500 g-min. Supernatant was poured through cheesecloth and was recentrifuged at 6500 g-min. The low-speed supernatant was centrifuged at 300 000 g-min. At this stage chromaffin granules containing DBH are in the pellet and PNMT as a soluble enzyme remains in the supernatant.

The supernatant containing PNMT was stabilized with dithiotreitol (final conc. 1 mM) and was precipitated with ammonium sulphate. The 0-30 % precipitate was discarded. The 30-60% precipitate was dialysed and chromatographed on a Sephacryl S-200 column. The column was eluted with 50 mM Tris-buffer (pH 7.4).The fractions from the column which were found to contain the highest PNMT activity were pooled and concentrated with an Amicon pressure dialysis cell. PNMT activity was assayed by a modification of a previously described procedure (Axelrod, 1961).

The chromaffin granules containing DBH were isolated further as described before (Smith & Winkler 1967). The pellet of chromaffin granules was frozen and thawed twice to lyse the granules. This lysate was centrifuged at 600 000 g-min. Supernatant was chromatographed on a concavalin A-Sepharose 4 B column as described before (Rush et al, 1974). The active fractions were pooled, dialysed and concentrated. DBH activity was assayed with the Nagatsu-method (Nagatsu & Udenfriend, 1972).

The simultaneous purification of DBH and PNMT lowered the yeald of enzymes when compared to the original purification procedures. However, since these two enzymes were needed the rapid gathering of two enzymes at the price of lowered yeald was considered worth-while. These enzyme fractions do not contain monoamine oxidase activity, but pargyline should be added to the incubation mixtures to avoid this source of error. Repeated freezing and thawing of either enzyme results in about 10 % reduction in enzyme activity. This was avoided by storing the enzymes in small (1-2 ml) samples. The enzymes were stable for years at -60^{o}C.

My sincere thanks are due to Dr J.P.Pispa for innumerable discussions about the details of the purification procedure.

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EVIDENCE FOR THE PRESENCE OF PRESYNAPTIC FACILITATORY β-ADRENOCEPTORS ON RENAL NORADRENERGIC NERVES

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It is reported that β-adrenoceptor agonists cause an increase in the stimulus-induced release of noradrenaline (NA) by activating presynaptic β-adrenoceptors (Langer, 1980). While presynaptic β-adrenoceptors are located on noradrenergic nerves innervating several tissues, the presence of these facilitatory receptors on renal noradrenergic neurons has not been demonstrated. Therefore, the present study was performed to determine the presence and functioning of presynaptic β-adrenoceptors in the isolated perfused rat kidney.

The right kidney was isolated and perfused at 6 ml/min with Krebs-Ringer solution. NA storage sites were labelled with ℓ-^{3}H-NA. The effects of β-adrenoceptor agonists, isoprenaline and salbutamol on ^{3}H-NA release during periarterial nerve stimulation was studied at stimulation frequencies of 0.5 and 2 Hz. Two control stimulations were applied for each of the stimulation frequencies and a ratio calculated as shown in Table 1. Increasing concentrations of isoprenaline and salbutamol when perfused through the kidney, caused an increase in the stimulus-induced release of ^{3}H-NA at 0.5 and 2 Hz. However, the facilitatory effect of salbutamol was concentration dependent and more pronounced than isoprenaline (Table 1). In addition, the maximum facilitatory effect of both these agents on ^{3}H-NA release was observed at 0.5 Hz (Table 1).

Table 1 Effects of isoprenaline (ISO) and salbutamol (SAL) on stimulus-indued release of ^{3}H-noradrenaline in the isolated perfused rat kidney

Drug	Stimulus Frequency	Control 2 / Control 1	[1x10^{-10}M] / Control 1	[1x10^{-8}M] / Control 1	[1x10^{-6}M] / Control 1
ISO	0.5 Hz	1.04±0.08	1.53±0.26	2.01±0.17	1.28±0.03
	2.0 Hz	1.02±0.02	1.02±0.03	1.28±0.06	1.33±0.08
SAL	0.5 Hz	1.03±0.08	1.33±0.15	1.71±0.10	2.47±0.16
	2.0 Hz	1.01±0.04	1.02±0.03	1.34±0.05	1.58±0.12

Additional experiments were performed at 0.5 Hz to assess the involvement of β-adrenoceptors in the facilitatory action of salbutamol. Perfusion of the kidney with ℓ-propranolol (10^{-9} to 10^{-5}M) did not cause any changes in the stimulus-induced release of ^{3}H-NA. However ℓ-propranolol (10^{-7}M) antagonized the facilitatory action of salbutamol on ^{3}H-NA release during periarterial nerve stimulation.

These results demonstrate the presence of presynaptic facilitatory β-adrenoceptors on renal noradrenergic nerves. The facilitation of stimulus-induced release of NA is better observed during low frequency of nerve stimulation and these presynaptic β-adrenoceptors may be of β_2-type as reported for human vasoconstrictor nerves (Stjarne and Brundin, 1976).

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5-HYDROXY-6-METHYL-di-n-PROPYLAMINOTETRALIN (DK-118), A POSSIBLE PRO-DRUG

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Several series of compounds have been reported to be metabolized to active dopamine-receptor agonists. In the aminotetralin series an excellent example would be the dibenzoate esters of ADTN (Horn et al 1979). Metabolic activation of a compound offers several possibilities for modifying biological activity. These would include absorption, distribution and varying the type of dopamine-receptor involvement. Evidence indicates that dopamine-receptors are not a homogenous entity. Possible involvement of different dopamine-receptors by DK-118 and its metabolic products was evaluated.

The ability of DK-118 to lower arterial pressure, induce bradycardia and inhibit tachycardia induced by postganglionic cardioaccelerator nerve stimulation was evaluated in cats anesthetized with pentobarbital sodium. The adrenergic nerve terminal inhibiting action of DK-118 was also evaluated *in vitro* using cat atria and field stimulation. Two metabolic inhibtory agents (metyrapone, 20 mg/kg and SKF 525-A 30 mg/kg) were used with the *in vivo* experiments.

In anesthetized cats, DK-118 was found to produce a potent (ED50-0.025μM/kg) and long lasting inhibition of cardiac sympathetic nerve transmission and this inhibition appears to be mediated by presynaptic dopamine receptors (Verimer et al 1981). The onset of action required at least 10 minutes with maximal inhibition of transmission being observed in 20-30 minutes following intravenous administration. The delayed onset of action may suggest metabolic activation of DK-118. This possibility was supported by the observation that metyrapone and SKF-525-A antagonized significantly the ability of DK-118 to inhibit responses to stimulation of the right cardioaccelerator nerves. These agents do not antagonize inhibitory action of N-*di*-propyldopamine. However, the metabolic inhibitors do not antagonize the ability of DK-118 to induce bradycardia and decrease arterial pressure. With isolated cat atria DK-118 was found to be inactive as an inhibitor of tachycardia induced by field stimulation. In these preparations apomorphine is active (ED50-0.08μM). With isolated cat atria, DK-118, N-*di*-C_2H_5 and N-*di*-CH_3 analogs (10 μM) were found to antagonize neuronal inhibitory actions of apomorphine.

These studies demonstrate that DK-118 is a potent, long-acting inhibitor of adrenergic neuronal transmission. Inhibition is antagonized by dopamine-receptor blocking agents and mixed function oxidase-inhibitors. Since the hypotension and bradycardia induced by DK-118 are not modified by metabolic inhibitors but are blocked by dopamine-receptor blocking agents perhaps these cardiovascular responses are induced at some site other than the adrenergic nerve terminal. DK-118 may interact with dopamine-receptors to lower arterial pressure while metabolic alteration of the parent compound is necessary before inhibition of adrenergic nerve terminals can occur. This study also adds further support to the suggestions that various dopamine-receptor types may modify cardiovascular function.

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GABA REDUCES THE EVOKED NEURONAL RELEASE OF (^{3}H)-NORADRENALINE FROM RAT ANOCOCCYGEUS MUSCLE: ANTAGONISM BY 5-AMINOVALERATE

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GABA and baclofen depress the evoked release of [^{3}H]-noradrenaline from rat atrium through an interaction with bicuculline-insensitive receptors, which have been termed $GABA_B$ sites (Bowery et al, 1981). Our own studies on the rat anococcygeus muscle have indicated that these receptors are also present on the adrenergic nerve terminals of this preparation. GABA and (-)-baclofen were equipotent in depressing the twitch responses of the electrically-stimulated muscle, and their effects were not inhibited by the traditional GABA antagonists, bicuculline and picrotoxin. However, 5-aminovalerate proved to be an effective, albeit rather weak, antagonist at this site (Muhyaddin, Roberts and Woodruff, submitted).

In the present study we have investigated the effects of GABA and (±)-baclofen on the overflow of [^{3}H]-noradrenaline during electrical nerve stimulation of the anococcygeus muscle. Male Wistar rats were killed and both muscles were dissected out and incubated with 0.5 μM 1-[7,8-^{3}H]-noradrenaline in Krebs-Henseleit solution at 20 °C for 30 min. The muscles were suspended in Krebs medium containing cocaine (4.4 μM), phentolamine (4 μM) and ascorbate (0.1 mM) in 4 ml organ baths. The medium was collected and assayed for released radioactivity every 10 min, and the preparations were stimulated at a frequency of 1 Hz, 0.1 msec pulse duration at a supramaximal voltage for 9 min in every 30 min period. In each experiment the tissues were stimulated three times and GABA and baclofen were added 1 min, and 5-aminovalerate, 5 min before periods S_2 and S_3 of electrical stimulation. GABA was found to depress the evoked, but not the basal release of [^{3}H]-noradrenaline in a dose-dependent manner (10^{-7} -10^{-4} M) to a maximum of 58 ± 3 per cent inhibition (mean ± S.E.M. of 6 experiments), and showed an EC_{50} of approx 0.6 μM. (±)-Baclofen inhibited the evoked release to a similar extent. Inclusion of 5-aminovalerate in the medium resulted in an inhibition of the response elicited by 10 μM GABA. The drug alone did not influence either the spontaneous or evoked [^{3}H]-noradrenaline release. Since for control experiments (stimulation alone) S_2/S_1 and S_3/S_1 ratios did not differ significantly from each other, it was possible to combine all drug data obtained in each experiment (Table 1).

Table 1 Effect of GABA (10 μM) on the evoked release of [^{3}H]-NA and its antagonism by 5-aminovalerate

Treatment	Stimulus ratio	n
Control S2/S1	1.058 ± 0.028	5
Control S3/S1	0.992 ± 0.05	5
GABA S2,S3/S1	0.418 ± 0.03	6
GABA + 0.5 mM 5-aminovalerate S2,S3/S1	0.547 ± 0.04 *	5
GABA + 1.0 mM 5-aminovalerate S2,S3/S1	0.807 ± 0.07 ***	7

* $P < 0.05$; *** $P < 0.001$ as compared with GABA alone, by t-test.

The results presented here are in good agreement with those we have found for the drugs on the twitch response of the anococcygeus muscle.

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EFFECT OF PARASYMPATHECTOMY ON (^{3}H)-QNB BINDING IN RAT PAROTID SALIVARY GLANDS

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Sympathetic denervation of the salivary glands results in an increased responsiveness to adrenoceptor agonists and this has been explained, at least in part, by an increase in α- and β-adrenoceptor number (Pointon and Banerjee, 1979). Similarly, parasympathetic denervation (Px) results in a supersensitivity to cholinergic muscarinic agonists. It seems reasonable to suggest that this increase in sensitivity could result from an increase in the number of muscarinic receptors. This possibility was investigated using the radioligand [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB).

The auriculo-temporal nerve to the parotid gland was sectioned unilaterally in pentobarbitone-anaesthetised rats (60 mg/kg i.p.). The contralateral nerve was not sectioned, thus each animal provided a control and denervated gland. Six weeks after denervation the parotid glands were removed, cleaned, weighed and membranes prepared by standard techniques. Saturation curves for [^{3}H]-QNB binding (0.1 - 2.0 nM) were constructed with membranes from control and Px glands using atropine (10 μM) to determine the specific binding. Assays were performed in triplicate at 37 °C for 30 min and bound radioactivity separated by filtration through GF/C filters. The experiments were performed with three groups of five animals.

Binding of [^{3}H]-QNB was saturable in both control and Px membranes, Scatchard analysis of the specific binding revealed a single population of [^{3}H]-QNB binding sites. Following Px there was no difference in the affinity, the values for Kd being 0.76 ± 0.26 and 0.79 ± 0.1 nM in Px and control membranes respectively. Similarly, there was no change in Bmax when expressed as sites/mg membrane protein (67.7 ± 11.9 and 95.2 ± 33.5 pmole/mg for control and Px). However, as reported previously (Poat and Templeton, 1981) Px causes a fall in gland weight and a fall in membrane protein yield. Thus, when [^{3}H]-QNB binding was corrected to give an estimate of the number of sites/gland it is clear that Px causes a substantial reduction (801.7 ± 158 and 232.7 ± 137 pmole/gland, for control and Px).

This effect of Px, reducing the number of [^{3}H]-QNB sites/gland is unexpected in view of the large increase in sensitivity to cholinergic agonists displayed by the denervated gland (Ekstrom, 1980). The development of this supersensitivity may therefore involve other mechanisms including more effective stimulus-secretion coupling leading to increased Ca^{++} flux or increased 2nd messenger generation.

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A COMPARISON OF THE PROFILES OF DESIPRAMINE AND THE α_2-ADRENOCEPTOR ANTAGONIST RX781094 IN THE PITHED RAT

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Both prejunctional α_2-adrenoceptor antagonists and inhibitors of neuronal uptake have the potential to increase the synaptic concentration of noradrenaline (NA). It is likely therefore that some aspects of their pharmacological profiles will be similar. This possibility has been explored by comparing the effects of a new potent, selective α_2-adrenoceptor antagonist RX 781094 (Chapleo et al., 1981) and the uptake inhibitor desipramine (DMI) in the pithed rat.

Contractions of the anococcygeus muscle elicited by electrical stimulation (1-6 Hz, 40v, 0.5 ms, 20s) of the spinal sympathetic outflow of pithed rats were potentiated both by RX 781094 (0.01-1 mg/kg,i.a.) and by DMI (0.01-0.1 mg/kg,i.a.). Contractions of this tissue induced by either i.a. (femoral) NA (0.3 ug/kg) or phenylephrine (PHE 1 ug/kg) were potentiated by these doses of DMI, but unaffected by RX 781094 in doses up to 1 mg/kg, i.a. The potentiation of the responses to nerve stimulation produced by a combination of DMI (30 ug/kg, i.a.) and RX 781094 (100 ug/kg, i.a.) was significantly greater than that observed with DMI alone. The inhibitory effects of guanabenz (30 ug/kg,i.v.) on stimulation (1 Hz, 40v, 0.5 ms for 20s every 2 min)-induced contractions of the anococcygeus muscle were fully reversed by both RX 781094 (3-100 ug/kg,i.v.) and DMI (30-100 ug/kg,i.v.).

Stimulation (6Hz, 40v, 50 us for 2s every 30s)-induced contractions of the vas deferens were unaffected or slightly potentiated by RX 781094 in doses up to 1 mg/kg, i.v. In contrast, the twitch response of this tissue was progressively inhibited by DMI (ID_{50} = 0.67±0.2 mg/kg, i.v.); this inhibition was fully reversed by RX 781094 (0.1 mg//kg, i.v.). In experiments in which the twitch response was completely inhibited by clonidine (100 ug/kg, i.v.) RX 781094 fully reversed this inhibition; its ED_{50} value being 24±1 ug/kg, i.v. However, DMI (0.01-1 mg/kg, i.v.) failed to antagonise the inhibitory effects of clonidine.

These results demonstrate that in the anococcygeus muscle it is difficult to differentiate between selective α_2-adrenoceptor antagonists and uptake inhibitors unless their effects on responses to exogenously applied agonists are also studied. In the vas deferens, however, their profiles are quite dissimilar. The inhibitory effect of DMI in this tissue and its reversal by RX 781094 suggest that when synaptic levels of NA are increased by inhibition of neuronal uptake, stimulation of prejunctional α_2-adrenoceptors occurs. The inhibition of locus coeruleus firing rate following acute DMI administration is also mediated by activation of α_2-adrenoceptors (Svensson and Usdin, 1978). This acute inhibitory effect may explain why NA uptake inhibitors do not produce rapid relief of mental depression, but do so only on chronic administration following the development of α_2-adrenoceptor subsensitivity (Crews and Smith, 1978).

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THE DIFFERENTIAL EFFECT OF ACIDOSIS ON α_1-ADRENOCEPTOR-MEDIATED PRESSOR RESPONSES

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α_1- and α_2-Adrenoceptor-mediated pressor responses, in the pithed rat, may result from different biochemical mechanisms: (1) α_1 Responses are implicated in 'Ca^{++}-gating' and α_2 with inhibition of adenylate cyclase (Fain & Garcia-Sainz, 1980). (2) The pressor response to methoxamine, an α_1-agonist, is resistant to Ca^{++}-entry blockers whereas the response to B-HT 920, an α_2-agonist, is antagonised (van Meel et al, 1981). (3) Respiratory acidosis increases the potency of α_2-agonists but decreases that of α_1-agonists (Flavahan & McGrath, 1981). We have now investigated whether this effect of acidosis might involve either of these reported differences in the biochemical mechanisms set in train by these receptors.

Male Wistar rats (250-275g) were pithed and respired with O_2. Carotid arterial pressure was monitored. Drugs were injected via a jugular vein. Respiratory acidosis was induced as described previously (Flavahan & McGrath, 1981). Xylazine, a selective α_2-agonist, evoked a pressor response which, like that to B-HT 920, was antagonised by the Ca^{++}-entry blockers nifedipine (0.3mg/kg) or bepridil (25 mg/kg). Pressor responses to phenylephrine were resistant to these blockers.

The pressor response to amidephrine, a selective α_1-agonist, consisted of two components: an early rapid peak was resistant to nifedipine (0.3 mg/kg) or bepridil (25mg/kg) and had a time-course similar to phenylephrine's; a second, slower component was antagonised by the Ca^{++}-entry blockers and had a time-course similar to xylazine's. These two components were antagonised to an equal extent by prazosin (0.01-1mg/kg) and were unaffected by rauwolscine (1mg/kg). Therefore, both components were due to stimulation of α_1-adrenoceptors.

Amidephrine's late, Ca^{++}-entry blocker-sensitive component was also similar to xylazine's response in being greater following respiratory-induced acidosis. However, the initial component, like phenylephrine's effect, was decreased.

In conclusion, α_1- and α_2-adrenoceptor stimulation can both increase vascular resistance by mechanisms which are sensitive to Ca^{++}-entry blockers, presumably by initiating Ca^{++} influx. In addition, α_1-agonists can produce a response which is resistant to these blockers, presumably by releasing Ca^{++} from intracellular stores. The failure of phenylephrine or methoxamine to stimulate Ca^{++} influx may be due to the brevity of their action but we cannot exclude a difference at the level of the receptor.

The effect of acidosis on the agonists' responses appears to depend on the biochemical mechanism of the response rather than an effect at the receptor per se. Those mediated by stimulation of Ca^{++} influx are increased by acidosis whereas those requiring intracellular Ca^{++} are decreased.

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PRE- AND POSTSYNAPTIC EFFECTS OF CIRAZOLINE IN THE PITHED RAT AND RAT VAS DEFERENS

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The imidazoline derivative cirazoline is a potent α_1-adrenoceptor agonist (Roach et al,1978), but its action at presynaptic α_2-adrenoceptors has been reported both as agonism (cat spleen: Dubocovich et al,1980) and antagonism (rat heart and vas deferens: Cavero et al,1982). Since these conflicting results could be explained if cirazoline were a partial agonist at α_2-adrenoceptors, we have examined this possibility employing the pithed rat and rat isolated vas deferens.

In pithed rats, presynaptic α-agonism by cirazoline was assessed against the cardioaccelerator response to a single stimulus pulse, and postsynaptic α-agonism as the rise in diastolic blood pressure (DBP) produced by the drug (Docherty & McGrath,1980). Cirazoline caused dose-dependent inhibition of the cardioaccelerator response and dose-dependent pressor responses. The presynaptic ID_{50} of cirazoline (dose producing 50% inhibition of cardioaccelerator response) was 7.6 ± 2.1μg/kg, which in the presence of yohimbine (1mg/kg) was shifted to 85 ± 24 μg/kg. The dose of cirazoline producing a rise in DBP of 50mmHg (measured 5 min after injection) was shifted in the presence of prazosin (1mg/kg) from 35.5 ± 6.7 μg/kg to > 1mg/kg, and by yohimbine (1mg/kg) to 310 ± 140μg/kg. The experimental protocol employed tends to overestimate presynaptic potency: the postsynaptic/presynaptic dose ratio obtained for cirazoline was 4.7 compared with 26.9 for clonidine showing that clonidine has greater presynaptic selectivity. Cirazoline (0.1 & 1mg/kg) failed to reverse the clonidine-induced inhibition of the cardioaccelerator response to stimulation at 5Hz for 4sec, and, in the absence of clonidine, cirazoline itself caused an inhibition. Hence α_2-adrenoceptor agonist but not antagonist properties of cirazoline could be demonstrated.

In the field stimulated prostatic portion of the rat vas deferens (MacDonald & McGrath,1980), cirazoline (10-1000nM) caused a concentration-dependent potentiation of the contractile response to a single stimulus pulse. The inhibition by clonidine (10nM) of the contraction to a single pulse was completely reversed by cirazoline 100nM, and cirazoline 1μM potentiated the response above pre-clonidine levels. However, in the presence of prazosin (30nM) cirazoline 100nM was ineffective, cirazoline 1μM completely reversed the inhibitory effect of clonidine, and cirazoline 10μM potentiated the response above pre-clonidine levels. Hence, the apparent antagonism of the inhibitory effects of clonidine was by postsynaptic α-adrenoceptor agonism by cirazoline.

In conclusion, cirazoline is an agonist at postsynaptic α_1- and presynaptic α_2-adrenoceptors in the pithed rat and rat isolated vas deferens; no evidence was found for α_2-antagonism by cirazoline.

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EFFECTS OF FLUNARIZINE ON CALCIUM DEPENDENT CONTRACTIONS OF VASCULAR AND VAS DEFERENS SMOOTH MUSCLE

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Flunarizine is a vasodilator having greatest activity on the basilar artery and least on spontaneous contractions in the portal vein; it has little effect on the myocardium or uterus (Van Nueten et al., 1978; Nakayama & Kasuya, 1980). In rat aorta and mesenteric artery, flunarizine has been shown to inhibit noradrenaline stimulated ^{45}Ca uptake (Godfraind & Dieu, 1981).

Rabbit portal vein, aortic strips, mesenteric artery strips and rat vasa deferentia were suspended in Krebs-Hanseleit solution of the following composition: Na^+ = 144, K^+ = 5.8, Ca^{2+} = 2.5, Mg^{2+} = 1.2, HCO_3^- = 25, $H_2PO_4^-$ = 1.2, SO_4^{2-} = 1.2, Cl^- = 128.6, glucose = 11.1 mM, bubbled with 95% O_2:5% CO_2, maintained at 36-38°C and tension was recorded isometrically. Contractions produced by KCl in the vascular muscles (using the EC90 for each) were inhibited by flunarizine, approximately equieffective concentrations giving more than 90% inhibition were: aorta 2.1 μM, mesenteric artery and portal vein 0.21 μM. The effect of flunarizine developed progressively over 3-4 hours, giving a linear relationship between log % inhibition and time. In the vas deferens, concentrations of flunarizine (15 min contact) required for 50% inhibition of KCl 160 mM contractions were: tonic component 2.79 ± 1.03 μM and phasic component 27.9 ± 7.5 μM. Flunarizine, unlike other calcium channel inhibitors, selectively inhibited the tonic phase, over its entire concentration range. No further inhibition of the tonic component occurred when incubation was prolonged to 60 min, but inhibition of the phasic component was greater. Rhythmic contractions induced by methoxamine 8.1 μM or $BaCl_2$ 1 mM were reduced in size by flunarizine with little effect on their frequency. Methoxamine-induced rhythmic contractions were more sensitive (IC50 = 15.9 ± 5.95 μM) than were $BaCl_2$ induced rhythmic contractions (IC50 = 54.5 ± 20 μM). The maximal effect at each concentration was produced in 15 - 20 min. Flunarizine had no local anaesthetic activity on rat phrenic nerve below 40 μM.

The study has revealed marked differences in sensitivity for different agonists and different tissues. In general, vascular smooth muscle was more sensitive than vas deferens, and contraction produced by sustained depolarization was more sensitive than that produced by spikes. The selective effect of flunarizine on methoxamine induced rhythmic contractions was not seen with verapamil, methoxyverapamil or nifedipine, and may indicate that methoxamine and barium open different Ca channels. The time course of action of flunarizine on vascular smooth muscle was slower than on vas deferens, despite binding to microsomal fractions of rat aorta being complete in 4 min (Godfraind & Morel, 1981). In the 3 vascular muscles, the rate of development of block was similar for equieffective concentrations.

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PHARMACOLOGICAL ANALYSIS OF THE CONTRACTILE EFFECT OF ISOPRENALINE ON THE RAT ISOLATED SEMINAL VESICLE

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Although isoprenaline is usually considered to have almost no action on alpha adrenoceptors, sporadic reports in the literature describe a contractile effect of the drug in isolated tissues containing such receptors (Thompson, 1958; Day & Dixon, 1971). Recently, Spedding (1980) reported a phentolamine sensitive contraction of the rat vas deferens by isoprenaline but could not obtain complete concentration-response curves because of the low sensitivity of the preparation.

In this presentation we describe complete concentration-response curves for a contractile effect of isoprenaline on the isolated rat seminal vesicle which, to our knowledge, has not been described previously and present a quantitative pharmacological analysis of this response.

Individual horns of seminal vesicles removed from young (125-150g) rats were mounted in a 5ml jacketed organ bath in a continuousflow (15ml/min) of Krebs bicarbonate solution containing (±) propranolol(0.85μM) and normetanephrine (1.0μM) to block beta-adrenoceptors and extraneuronal uptake respectively. Contractions were recorded by means of an isotonic force-displacement transducer.

Concentration-response curves for isoprenaline (10^{-5}M to 10^{-2}M) were typically sigmoidal and values (mean ± s.e.mean) for slope and PD_2 computed by regression line analysis (over the range 20 - 80% of the maximum response) of individual curves were 55.8 ± 3.35 & 3.45 ± 0.06 respectively (n=10).

Phentolamine caused a parallel shift of the curves to the right. Using paired preparations, corrected dose-ratios for three different concentrations of phentolamine (10^{-8}M, 10^{-7}M, & 10^{-6}M) were determined in triplicate. Arunlakshana & Schild (1959) plots of the data were linear and had a slope, significantly greater than zero and not significantly different from unity ($P < 0.001$). The PA_2 and slope values for phentolamine were 8.03 ± 0.02 & −0.99 ± 0.005 respectively. These values are in good agreement with the PA_2 values for phentolamine against noradrenaline in the same tissue (our unpublished observations) and with those reported in other tissues containing alpha receptors (Apperley et al, 1976; Furchgott, 1967).

Reciprocal analysis of the contractile effect of isoprenaline alone and in the presence of phentolamine yielded straight lines with different slopes but a common intercept at a point corresponding to infinite dose.

In receptor protection experiments, a high concentration (10^{-3}M) of isoprenaline protected noradrenaline against blockade both by phentolamine (10^{-6}M) and phenoxybenzamine (10^{-8}M).

Our results suggest that isoprenaline causes a contraction of the rat seminal vesicle by activating post-junctional alpha-adrenoceptors which are identical with the contractile receptors for noradrenaline in the same tissue and in other tissues containing alpha receptors.

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THE LACK OF SPECIFICITY OF 4-METHYLPYRAZOLE IN POTENTIATING THE EFFECTS OF ETHANOL IN VIVO

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Pyrazole and its derivatives have been used as liver alcohol dehydrogenase (LAdH) inhibitors for many years, and pyrazole has found clinical use in the treatment of methanol and ethylene glycol poisoning (Van Stee et al., 1975; Blomstrand et al., 1979). The relative specificity of pyrazole towards LAdH has always been assumed since the work of Reynier (1969), and the potentiation of general depressant drug effects by this compound has been taken to imply the involvement of LAdH in the metabolism of the drug (e.g. Schultz & Weiner, 1979). However, in studies on the interaction between ethanol and chloral hydrate (Owen & Taberner, 1980), pyrazole was found to potentiate trichloroethanol which itself is an LAdH inhibitor not metabolized by the enzyme. Although in recent years pyrazole has been found to be toxic at doses which inhibit LAdH, 4-methylpryazole has been increasingly used as a more potent and specific LAdH inhibitor (Magnusson et al., 1972). We have therefore examined the effects of pretreating mice with 4-methylpyrazole subcutaneously (s.c.) on the duration of the loss of righting reflex following the subsequent administration of various general depressant drugs.

Adult C57BL mice of either sex were used throughout. Drugs were made up in 0.9% (w/v) saline and injected i.p. except where indicated. Halothane, ether and temazepam were suspended in arachis oil.

4-Methylpyrazole (1 mmol/kg s.c.) 60 min prior to drug treatment significantly prolonged the duration of loss of righting reflex following ethanol (4 ml/kg), chloral hydrate (300 mg/kg), 2,2,2-trichloroethanol (250 mg/kg), pentobarbitone (50 mg/kg), temazepam (100 mg/kg) and halothane (1 g/kg), but had no effect on the duration of loss of righting reflex following barbitone (200 mg/kg) or ether (1 g/kg). 4-Methylpyrazole alone had no discernable depressant activity at the dose used.

Of the drugs listed above only ethanol and chloral hydrate are metabolised by LAdH, and barbitone, ether and halothane are largely excreted unchanged. It is therefore apparent that the potentiation of some depressant drugs by 4-methylpyrazole does not necessarily involve LAdH inhibition. 4-Methylpyrazole should therefore be regarded with suspicion in any studies involving the role of LAdH in general depressant drug metabolism.

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THYROID CONTROL OF HEPATIC DRUG METABOLISM IN THE RAT

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Recent evidence suggests that the thyroid gland may be involved in the control of cytochrome P-450-mediated drug metabolism in the rat liver. Thyroidectomy (TX) (Kato & Gillette, 1965), thyroxine (T4) treatment (Rumbaugh et al, 1978) and propylthiouracil (PTU) treatment (Moreno et al, 1981) all give effects on drug metabolism. The effects, however, are not consistent. This project was designed to investigate the possible reasons for the inconsistency of thyroid effects on drug metabolism.

The effects of TX and T4-replacement therapy were tested on the metabolism of lidocaine, imipramine and diazepam by microsomal fractions of male and female rat liver. The radiometric assays were as previously described (Skett et al, 1980). The results are as shown in Table 1.

Table 1. Effect of Thyroidectomy and Thyroxine-replacement therapy on Hepatic Drug Metabolism

Group \ Enzyme	Cyt P-450 (nmol/mg)	LIDOCAINE 3-OHase	LIDOCAINE N-De-ethylase	IMIPRAMINE N-Oxidase	IMIPRAMINE N-De-methylase	IMIPRAMINE 2-OHase	DIAZEPAM 3-OHase	DIAZEPAM N-De-ethylase
Control ♂ (A,8)[1]	100± 64[2]	100±29	100±34	100± 62	100±18	100±14	100±51	100±46
TX ♂ (B,4)	147± 46	202±80	257±71	60± 26	88±15	143±15	95±41	94±36
TX+T4 ♂ (C,4)	280±108	138±44	161±20	50± 14	84±20	142±29	175±28	162±17
Sign.[3]	C B A	B C A	B C A	C B A	C B A	A C B	B A C	B A C
Control ♀ (D,8)	100± 20	100±33	100±43	100± 31	100±26	100±33	100±37	100±33
TX ♀ (E,4)	78± 13	150± 4	171±50	246± 23	153±28	133±33	135±43	137±41
TX+T4 ♀ (F,4)	57± 16	100±25	131±12	418±309	173±40	140±33	130±14	146±12
Sign.[3]	F E D	D F E	D F E	D E F	D E F	D E F	D F E	D E F

1. group letter, no. of animals in group; 2. % of control ± 1 S.D.;
3. means arranged in rank order, those underlined are not significantly different ($p > 0.05$) according to Duncan's Multiple Range Test.

It is seen that the effects of TX are substrate- and sex-dependent e.g. TX causes an increase in lidocaine metabolism but little effect on diazepam and imipramine metabolism in the male whereas a marked increase in imipramine metabolism is seen in the female. A partial reversal of the effects of TX can be achieved by T4 but in some cases T4 potentiates the effect of TX.

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APPLICATION OF HPLC AND NMR TO THE ONE-STEP SYNTHESIS OF AUTHENTIC NORADRENALINE 3- AND 4-O-SULPHATES

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Several reports confirm that both circulating and excreted catecholamines are found largely as their sulphate conjugates (Jenner & Rose, 1974; Kuchel et al., 1978; Kuchel et al., 1980). In spite of the obvious importance of sulphation to the disposition of, for example, noradrenaline, no direct assay of these conjugates has been developed, current methods relying upon deconjugation and further assay of 'total' noradrenaline. This is thought to be because the authentic noradrenaline sulphates have not been available, although the definitive synthesis of these has been performed, but involves a complex seven-step procedure (Wang et al., 1972).

We have recently demonstrated however (Idle et al., 1982), that the analogous dopamine O-sulphates could be prepared in a simple one-step reaction, isolated by preparative h.p.l.c. and authenticated by 250 MHz n.m.r. techniques. Accordingly, we have applied similar methodology to the synthesis and characterisation of the noradrenaline O-sulphates.

Noradrenaline (2.5 g) was reacted with chlorosulphonic acid (10 ml) containing N, N-diethylaniline (1.5 ml) at 7°C for 30 min, poured into water (50 ml), $BaCO_3$ added until pH 5 was reached and then $BaSO_4$ removed by centrifugation. The supernatant was applied to a Dowex 50 X 8 column (H^+ form, 200-400 mesh; 580 X 16 mm) and eluted with water, collected in 10 ml aliquots. Fractions 11-20 (A) and 50-200 (B) were concentrated to 10 ml each and examined by h.p.l.c. (solvent: H_2O, column: Hypersil 5 ODS, 250 X 10 mm). Fraction A contained Products I and II (elution vols: 23 and 24 ml) and Fraction B largely Product I. These were isolated to >95% purity by preparative h.p.l.c. giving Product I (47 mg; m.p. 161-162 (d) °C; calc. for $C_8H_{11}NO_6S$: C, 38.55; H, 4.42; N, 5.62%; found: C, 38.64; H, 4.42; N, 5.50%) and Product II (63 mg; m.p. 164°C; calc. for $C_8H_{11}NO_6S$: C, 38.55; H, 4.42; N, 5.62%; found: C, 39.09; H, 4.54; N, 5.68%). Proton n.m.r. spectra of I and II in d_6-DMSO at 250 MHz confirmed them to be noradrenaline 4- and 3-O-sulphates respectively. In each case, three aromatic protons were seen, two doublets and a quartet as follows: I, 6.77 ($J_{o,m}$), 6.87 (J_m) and 7.15 (J_o); II, 6.83 (J_o), 6.97 ($J_{o,m}$) and 7.20 (J_m) δ ppm relative to TMS. Aliphatic protons of I and II gave a complex signal identical to noradrenaline, showing absence of side-chain sulphation.

Thus, a one-step synthesis, isolation and characterisation of noradrenaline 3- and 4-O-sulphates has been achieved. As stated previously (Idle et al., 1982), h.p.l.c. combined with n.m.r. presents the best opportunity for obtaining pure characterised synthetic sulphate conjugates of catecholamines and related compounds.

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AN HPLC ASSAY FOR ETHYLENEDIAMINE IN PLASMA AND URINE

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Ethylenediamine (EDA; 1,2-diaminoethane) is a strong base widely used in the chemical industry, in various topical pharmaceuticals and, in combination with theophylline, is found in aminophylline. EDA has a variety of biological actions, notably allergenicity, and enhances the metabolism of theophylline. Probably due to its chemical nature, which makes its analysis difficult, there is little information in the literature on the disposition of EDA. As part of our studies upon the biological fate of aminophylline we have developed a sensitive HPLC assay for EDA in plasma and urine, in which it is converted to its N,N'-di(m-toluoyl)-derivative.

1ml of plasma was spiked with 2µg cadaverine as internal standard, mixed with an equal volume of 10% trichloroacetic acid and centrifuged. 1ml of the supernatant was mixed with an equal volume of 10% Na_2CO, 50µl of m-toluoyl chloride in acetone (100mg/ml) added and the whole shaken for 12h. The reaction mixture was extracted with 10ml methylene chloride, the methylene chloride evaporated and the residue taken up in 50µl CH_3CN for HPLC.

The column was 100 x 5mm packed with ODS-Hypersil 5µ, with a mobile phase of 40% aqueous CH_3CN, flow rate 0.9ml/min, with u.v. detection at 254nm. In this system the N,N'-di(m-toluoyl) derivative of EDA had R_T 5min, and the corresponding derivative of cadaverine had R_T 7min.

For urine, 1ml aliquots were spiked with 5µg cadaverine, 1ml 5M-NaOH added and extracted with 10ml diethyl ether, to remove interfering compounds. The ether was discarded and 1ml aliquots of the aqueous phase were treated as described for plasma.

Calibration curves over the range 0.1-10µg/ml EDA in plasma and 1-10µg/ml EDA in urine were linear, reproducibility being ± 3% at 5µg/ml and ± 7% at 0.5µg/ml (n = 4).

The method has been applied to a study of the pharmacokinetics of EDA after the oral and i.v. administration of aminophylline to volunteers. After i.v. injection, plasma concentrations followed a 2 compartment model, with α $t_{\frac{1}{2}}$ ca. 10 min, β $t_{\frac{1}{2}}$ ca. 35min, and volumes of central and peripheral compartments were 0.2 and 0.13l/kg respectively. After oral administration, the peak plasma levels were 1/10 of those seen after i.v. injection, and the bioavailability was estimated to be 30%. Excretion of unchanged EDA in the 0-24h urine totalled 20% after i.v. and 5% after oral administration. All results represent the means of 3 subjects.

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