EFFECTS OF PROPHYLACTIC AND THERAPEUTIC DOSING OF NONSTEROIDAL ANTI-INFLAMMATORY DRUGS ON A 24 HOUR CARRAGEENIN PLEURISY

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We have shown previously a correlation between inhibition of polymorphonuclear cell (PMN) infiltration and exudate by nonsteroidal anti-inflammatory drugs (NSAIDs) in the 5h carrageenin-induced pleurisy in rats (Blackham & Owen, 1975). Recent evidence, however, suggests that NSAIDs are ineffective against the exudative response in the later stages (5-24h) of this model (Bradshaw et al, 1982; Harada et al, 1982). We have investigated these observations using different dosing regimens of some NSAIDs and of the dual cyclooxygenase/lipoxygenase inhibitor BW 755C.

Female, Cobb Wistar rats (200-240g) (N=6 or more) were injected intrapleurally with 0.25ml of 1% Viscarin carrageenin. Drugs were given orally either 1h before, 5h and 21h after carrageenin ('prophylactic' regimen) or at 5h and 21h after carrageenin ('therapeutic' regimen). Pleural exudates were collected at 24h and the volumes recorded. Total and differential cell counts were performed.

Naproxen, indomethacin and ketoprofen enhanced exudate when given prophylactically and reduced mainly mononuclear cell infiltration (Table 1). Dosing therapeutically inhibited exudate and reduced the infiltration of both cell types to a similar extent. It is doubtful whether enhanced exudation was the result of a delay in the response, since exudate was still increasing at 24h. It is likely that the differential effects of the dosing regimens on cell populations were responsible for the exudative responses. BW755C resembled the effects of NSAIDs when given therapeutically, but also reduced exudate when administered prophylactically (Table 1). Inflammatory cell populations were affected similarly by prophylactic dosing of BW755C and NSAIDs. This suggests the partial involvement of lipoxygenase products of arachidonic acid as mediators of the 24h exudative response in prophylactically dosed animals.

Table 1 : Effects of NSAIDs on the 24h carrageenin pleurisy in rats

Drug	Dose (Regimen)	Exudate volume (ml)	Total cell count	PMNs	Mononuclear cells
Vehicle (0.05% Tween)	$1ml kg^{-1}$	3.5(2.1-4.6)	243(182-298) $x10^6$	147(109-190) $x10^6$	90(56-130) $x10^6$
			% changes		
Naproxen	$10mgkg^{-1}$(P)	+31*	-21*	-12	-42*
	$10mgkg^{-1}$(T)	-56*	-33*	-34	-22
Indomethacin	$3mgkg^{-1}$(P)	+37*	-28*	-10	-53*
	$3mgkg^{-1}$(T)	-27*	-23*	-19	-30
Ketoprofen	$10mgkg^{-1}$(P)	+48*	-36*	-17	-77*
	$10mgkg^{-1}$(T)	-39*	-28*	-27*	-30*
BW755C	$50mgkg^{-1}$(P)	-25*	-28*	-16	-43*
	$50mgkg^{-1}$(T)	-56*	-34*	-29	-37

Dosing Regimens: (P)=prophylactic; (T)=therapeutic (see text for explanation). Control (vehicle) group values represent means (N=18), ranges in parentheses. *$p<0.05$ compared to control animals. Student's two-tailed 't' test.

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CYCLIC GMP IN DIFFERENT BRAIN AREAS FROM EPILEPTIC GOLDEN HAMSTER

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Changes in cyclic GMP have been reported in both focal (Raabe et al. 1978) and generalised (Ferrendelli, 1976)experimentally induced epilepsy and in the amygdalae of rats following kindling (Blackwood et al., 1981). This study examines the concentration of cyclic GMP in different brain areas in epileptic hamster (cricetus auratus). In one group of hamsters intended for the study of some aspects of hybernation we observed that some of them presented spontaneous audiogenic epilepsy. We bred them and separated the descendants into two groups of siblings "epileptic" and "non epileptic" hamsters, all coming from "epileptic" parents. They were housed together and the noise in the environment was strictly controlled to avoid seizures during at least the week before the animals were killed. Immediately after decapitation different brain areas were dissected. The tissue from one side was immediately homogenized in ethanol and the tissue from the other side was sliced using a McIlwain chopper. The slices were incubated at 37°C for one hour at pH 7.4 in Krebs buffer (120 mM NaCl, 4.7 mM KCl, 25 mM $NaCO_3$, 1.2 mM KH_2PO_4, 1.0 mM $CaCl_2$, 2.3 mM$MgSO_4$, 10 mM glucose). The incubation was stopped by the addition of ethanol and immediate homogenization. The suspension was evaporated to dryness then resuspended in tris EDTA buffer for cyclic GMP estimation by radioimmunoassay (Radiochemical Centre, Amersham). The results in Table 1 show higher levels of cyclic GMP in most areas studied. The figures are 1 lower in the tissue homogenized immediately following extraction than in the tissue incubated for an hour (probably due to the post mortem changes which would settle during the incubation time) but the direction of the difference is the same.

Since cGMP is involved in membrane depolarisation and the subsequent release of neurotransmitter (Ferrendelli et al., 1976) the increase reported here may mediate an increased neuronal excitability. This could be secondary to the anatomical abnormalities of the astrocytes described for these animals by Coca et al. (1981).

Table 1: cGMP concentration (pmol x mg. prot.$^{-1}$) in different brain areas

	1h. Incubation		No Incubation	
	non-epileptic	epileptic	non-epileptic	epileptic
Frontal Cortex	1.98±0.28	4.50±0.19***	0.45±0.06	0.81±0.06**
Temporal Cortex	2.74±0.18	5.51±1.02*	0.65±0.11	1.09±0.14*
Striatum	1.90±0.08	4.46±0.13***	0.26±0.04	0.50±0.09*
Hippocampus	2.45±0.13	6.16±1.45*	0.24±0.08	1.66±0.50*
Colliculus Inferior	2.02±0.40	3.93±0.43*	0.63±0.08	1.64±0.23**
Tuberculus Acusticus	2.48±0.48	2.69±0.75	0.67±0.12	0.77±0.16

*, ** and ***: $p < 0.05$, $p < 0.01$, $p<0.001$ respectively (Student t-test).

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PROSTANOID PRODUCTION BY HUMAN PLATELETS: REDIRECTION BY THE THROMBOXANE SYNTHETASE INHIBITOR UK 37,248

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Collagen-induced platelet aggregation is associated with the release of arachidonic acid from phospholipids. The arachidonic acid is converted to endoperoxides and subsequently to thromboxane B_2 (TXB_2) and the prostaglandins (PGs) D_2, E_2 and $F_{2\alpha}$ (Smith et al, 1974; Oelz et al, 1977; Hamberg et al, 1974). Inhibition of thromboxane synthetase reduces TXB_2 production (Nijkamp et al, 1977). Data concerning the fate of the endoperoxides during such inhibition are, however, inconclusive. We have analysed the prostanoids formed from endogenous arachidonic acid during aggregation of human platelets in the presence and absence of the thromboxane synthetase inhibitor UK 37,248 (Randall et al, 1981).

Blood was collected from volunteers who had taken no drugs for at least 10 days and anticoagulated with 3.15% trisodium citrate (1 vol:9 vol blood). Platelet-rich plasma (PRP) was prepared by centrifugation at 200 g for 7.5 min at 20°C and platelet-poor plasma (PPP) by centrifugation at 1000 g for 30 min at 20°C. 5 ml of PRP were added to a Payton aggregometer cuvette at 37°C. UK 37,248 (final concentration 40 μM), or vehicle was added 1 min later and aggregation initiated with collagen (final concentration 2 μg/ml) at 3 min. 1 ml samples of PRP were removed 60 sec before the addition of collagen and 30, 60 and 180 sec after. The platelets were removed from the plasma by rapid filtration through glass fibre filters and the prostanoid content of the plasma analysed by gas chromatography negative ion chemical ionisation mass spectrometry (Waddell et al, 1983).

Table 1 Redirection of platelet prostanoid release by human platelets 3 min after stimulation with 2 μg/ml collagen

	Aggregation (%PPP)	TXB_2	PGD_2	PGE_2	$PGF_{2\alpha}$
Vehicle	68.4± 3.4 (6)	1414.80± 83.13 (6)	57.86± 6.46 (5)	48.43± 4.22 (6)	6.02± 0.41 (6)
UK 37,248	64.0± 3.0 (4)	9.90± 2.16 (4)	1220.79± 101.47 (4)	1063.55± 86.27 (4)	123.30± 13.51 (4)

Prostanoid (pmole) released by 10^9 platelets. Values are mean ± S.E.M.

Collagen induced a rapid release of prostanoids, mainly TXB_2. This was associated with the initiation of platelet aggregation. UK 37,248 did not significantly inhibit aggregation although TXB_2 release was inhibited by > 99% and release of PGD_2, PGE_2 and $PGF_{2\alpha}$ was increased by 21, 22 and 21 fold respectively (Table 1). The ratio of PGE_2 to PGD_2 was unchanged by UK 37,248. It is interesting that despite redirection of endoperoxides to PGD_2 there is little effect on collagen-induced aggregation of human platelets in vitro.

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COMPARISON OF THE RELEASE OF THROMBOXANE A_2 (TxA_2) FROM GUINEA-PIG ISOLATED PERFUSED WHOLE AND SUPERFUSED CHOPPED LUNGS

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Thromboxane A_2 (TxA_2) can be released from guinea-pig isolated lung, both chopped and whole, by a variety of stimuli (see Berti et al., 1981). In the present study we have compared the TxA_2-releasing action of a range of agents in both perfused whole lung and superfused chopped lung. The agents used were ovalbumen (OA), histamine (H), leukotriene D_4 (LTD_4), bradykinin (Bk), acetylcholine (Ach) and the stable TxA_2 mimetic U-46619 (Coleman et al., 1981).

Lungs were taken from guinea-pigs and either chopped and superfused (Coleman et al., 1980) or perfused through the pulmonary artery (Piper & Vane, 1969) at a rate of 5 ml/min with modified Krebs solution (Apperley et al., 1976). For experiments with OA, lungs were obtained from guinea-pigs sensitized with OA (2 mg i.p.) in Freund's complete adjuvent and saline (1:1) on days 1 and 5, animals being used on days 15-60. The lung effluent superfused a dog isolated saphenous vein for the detection of TxA_2 (Coleman et al., 1981). Drugs were administered cumulatively (Coleman et al., 1981) either into the fluid superfusing chopped lung or into the pulmonary artery of the perfused lung.

The results obtained are summarised in Table 1.

Table 1 Release of TxA_2 from guinea-pig whole and chopped lungs

Lung preparation	Doses causing TxA_2 release (mol)					
	OA+	H	LTD_4	Bk	Ach	U-46619
Whole	0.01-10	10^{-9}-10^{-7}	10^{-12}-$3x10^{-9}$*	10^{-11}-$3x10^{-9}$	> 10^{-7}	> $3x10^{-9}$
Chopped	0.1-10	>10^{-7}++	10^{-12}-$3x10^{-9}$	> $3x10^{-9}$	> 10^{-7}	> $3x10^{-9}$

+ dose in μgrams; ++ in 1 preparation only out of 4, slight release at 10^{-8} mol.
* effects sometimes seen with doses below 10^{-12} mol, see text

It will be seen that the six agents tested fell into three groups; those which release TxA_2 from both whole and chopped lungs (OA & LTD_4), those which release TxA_2 from whole but not chopped lungs (H & Bk) and those which release TxA_2 from neither (Ach & U-46619). OA caused TxA_2 release over the same dose-range in both whole and chopped lungs. The same was often true of LTD_4, however in some experiments with whole lungs, but never with chopped lungs release was obtained with doses of less than 10^{-12} mol, occasionally as low as 10^{-18} mol. The effects of LTD_4 on whole lungs are described in greater detail elsewhere (Coleman et al., 1983).

Two main points emerge from the present study. Firstly TxA_2 release is not secondary to smooth muscle contraction, since both Ach and U-46619 cause bronchoconstriction in the guinea-pig, but do not cause TxA_2 release. Secondly we have shown that whole and chopped lungs differ in their responsiveness to agents which release TxA_2.

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RELEASE OF THROMBOXANE A_2 FROM GUINEA-PIG ISOLATED PERFUSED WHOLE LUNG BY LEUKOTRIENE D_4, SOME UNEXPLAINED OBSERVATIONS

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In a previous communication we reported that leukotriene D_4 (LTD_4) can release thromboxane A_2 (TxA_2) from guinea-pig isolated whole and chopped lung preparations (Coleman et al., 1983). We also reported that, whilst LTD_4 consistently produced TxA_2 release from both whole and chopped lungs in doses of 10^{-12} mol and greater, in whole but not chopped lungs variable release was sometimes seen with much lower doses. We now describe the effects of LTD_4 on the whole lung in greater detail.

Guinea-pig isolated lungs were perfused and TxA_2 release detected using the dog isolated saphenous vein as described previously (Coleman et al., 1983). In our initial experiment we found that LTD_4 10^{-12}-$3x10^{-9}$ mol caused dose-related release of TxA_2, maximum contraction of the saphenous vein being obtained at about 10^{-10} mol. However, since the response to the lowest dose was 69% of the maximum, we went on to test lower doses. Surprisingly, whereas 10^{-14} mol LTD_4 produced a response 70.7 ($\pm$ s.e. mean 9.2, n=4)% of maximum, 10^{-12} mol gave only 46.2 ($\pm$ 9.0, n=6)% of maximum. Higher doses produced dose-related responses up to a maximum at 10^{-10} or 10^{-9} mol. Thus the dose-effect curve appeared to be biphasic. However, the first phase, but not the second, appeared to be subject to marked tachyphylaxis. Furthermore the first phase was not always obtained, only 50 out of 85 lungs responded to 10^{-14} mol LTD_4, whilst all responded to doses of 10^{-12} mol and greater. Nevertheless, in some experiments contractions of the saphenous vein were obtained with doses of 10^{-18} and 10^{-16} mol LTD_4.

Despite having studied this capricious sensitivity of whole lungs to very low doses of LTD_4 for over 12 months, we have been unable to relate it to any of the following : strain, batch, sex, age, weight, diet, sensitization to ovalbumen or state of health of the guinea-pigs, differences between different batches of LTD_4, pH of solutions, Ca^{++} concentration in the Krebs solution, perfusion rate or time of perfusion before challenge. We also note that other workers who have studied LT-induced TxA_2 release from guinea-pig lung have not reported this phenomenon (Berti et al., 1981; Piper & Samhoun, 1981).

In conclusion, our results are difficult to interpret, but are compatible with the hypothesis that LTD_4 can release TxA_2 from guinea-pig lung by two mechanisms. The first is sensitive to extremely low doses of LTD_4, is subject to tachyphylaxis and for as yet unexplained reasons does not always manifest itself. Furthermore this mechanism does not operate in chopped lungs (Coleman et al., 1983). The second mechanism is sensitive to higher doses of LTD_4, is not subject to tachyphylaxis and manifests itself consistently. This mechanism operates in both chopped and whole lungs.

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HYDROXY FATTY ACID LIPOXYGENASE PRODUCTS ARE PRESENT IN SUPERFICIAL PSORIATIC SCALE

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Psoriasis is a common inflammatory and proliferative skin disease, where an infiltration of neutrophils into lesional skin is observed. We have found that the potent chemotactic and chemokinetic dihydroxy-eicosatetraenoic acid, leukotriene B_4 (LTB_4), is released from abraded lesional psoriatic skin into chamber fluid (Brain et al., 1982). LTB_4 was initially detected by assessing chemokinetic activity in fractions separated by high performance liquid chromatography (HPLC). Using this method activity caused by material with a similar retention time to monohydroxy-eicosatetraenoic acids (monoHETEs) was also observed. We report here that a similar profile of activity is observed when superficial psoriatic scale extracts are separated by HPLC and we have identified some of the compounds that co-eluted in the monoHETE region.

Superficial scale was collected from untreated psoriatic lesional skin. Samples (approx. 150 mg, wet weight) were extracted by vortexing with a mixture of 0.1 M sodium acetate buffer, pH 3.5 and ethyl acetate. The ethyl acetate residue was partitioned between n-heptane and methanol to remove non-polar lipids. The methanolic extract was then applied to a straight phase HPLC column eluted with hexane/propan-2-ol/methanol/acetic acid (88:7:5:0.1) at 1 ml/min. Fractions of 1 ml were collected and chemokinetic activity was assessed in each by using leucocytes (70 - 80% neutrophils) in an agarose microdroplet assay (Smith & Walker, 1980). Chemokinetically active material with the same retention time as LTB_4 was seen, 435 (180 - 800) pg LTB_4 equivalents/100 mg scale (mean and range, n = 4). Comparable activity caused by material with a similar retention time to the monoHETEs was also observed in each case.

Subsequent extracts from scale (55 - 150 mg, wet weight) were subjected to HPLC in a system (two analytical silica columns in series, eluted with hexane/propan-2-ol/acetic acid, 96:4:0.1, by volume at 1 ml/min), which separated monoHETE compounds. Analysis of effluent fractions by gas chromatography-mass spectrometry revealed that the following monohydroxy compounds were present in psoriatic scale extracts: 12-HETE (confirming the findings of Hammarstrom et al., 1975), 15-HETE, 11-HETE, 9-HETE, 8-HETE, 5-HETE, 13-hydroxy-octadecadienoic acid (13-HODD) and 9-HODD. Of the HETE compounds, 12-HETE was present in highest concentrations, 1.31 (0.41 - 1.93) µg/100 mg scale (mean and range, n = 3). Both 13-HODD, 2.56 (1.12 - 4.60) µg/100 mg and 9-HODD, 0.27 (0.05 - 0.68) µg/100 mg (mean and range, n = 3), were also present in large concentrations. All measurements were uncorrected for recovery.

We have found that the HODD compounds (derived from linoleic acid) are chemokinetically inactive. Thus the chemokinetic activity caused by monohydroxy fatty acid-like material in psoriatic skin extracts is probably due to the presence of large amounts of 12-HETE. Most of the other monoHETEs also have chemokinetic properties, but their levels were at least twenty fold lower than those of 12-HETE.

It is possible that LTB_4 and monohydroxy fatty acids, or their hydroperoxy precursors, could be relevant to the pathogenesis of psoriasis.

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A COMPARISON OF THE EFFICACY OF INDOMETHACIN, DAZOXIBEN AND EP045 ON INTRAVASCULAR PLATELET AGGREGATION IN GUINEA-PIGS AND RATS

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When platelets and collagen interact, thromboxane A_2 (TXA_2) is produced via the prostaglandin endoperoxides by the mobilisation of arachidonic acid from platelet phospholipids. TXA_2 is a potent but labile vasoconstrictor and aggregatory substance that has been implicated in arterial thrombosis. When collagen is injected into anaesthetised animals, there is a fall in the circulating platelet count due to intravascular platelet aggregation (CIPA). To obtain an accurate and continuous measure of the circulating platelet count, the Technicon Autocounter was modified and used as described by Smith & Freuler (1973).

Collagen (40µg/kg i.v.) was injected into guinea-pigs or rats at 15 min intervals for several hours and a reproducible fall in platelet count was obtained. The effect of indomethacin (a cyclo-oxygenase inhibitor), dazoxiben (a thromboxane -synthetase inhibitor) and the thromboxane receptor blocker, EP045 (Jones & Wilson, 1981) on CIPA was studied by injecting the drug i.v. 5 min before injection of collagen. Blood samples were taken from a carotid artery (Mallarkey & Smith, unpublished) 1 min after injection of collagen for determination of thromboxane B_2 (TXB_2, sensitivity 0.3 ng/ml) and 6-keto prostaglandin $F_{1\alpha}$ (6-keto $PGF_{1\alpha}$, sensitivity 0.2 ng/ml) by radioimmunoassay.

In the guinea-pig, dazoxiben (10 mg/kg) produced a maximal inhibition of 54.3 ± 2.8% (n=5) of CIPA whilst a dose of 40 mg/kg was ineffective in the rat (n=5). In both species, plasma TXB_2 was reduced below the level of detection. 6-keto $PGF_{1\alpha}$, previously undetectable, was found in plasma from guinea-pigs (0.7 ± 0.1 ng/ml, n=4) and rats (0.8 ± 0.2 ng/ml, n=6). Near toxic doses of dazoxiben (80 & 160 mg/kg) produced only a weak inhibition of CIPA in the rat.

In the guinea-pig, indomethacin (1 mg/kg) produced 59.9 ± 5.2% (n=4) inhibition of CIPA whereas 8 mg/kg produced only 52.5 ± 1.6% (n=5) inhibition in the rat. TXB_2 was completely reduced and 6-keto $PGF_{1\alpha}$ was not detected in both species. EP045 reduced CIPA in the guinea-pig (5 mg/kg, 68.5 ± 2.0%, n=5) and in the rat (10 mg/kg, 33.9 ± 1.6%, n=4) when TXB_2 levels were not reduced.

The results suggest that conversion of platelet endoperoxide to TXA_2 is not essential for CIPA in the rat however such conversion is required by the guinea-pig. In the guinea-pig, indomethacin and EP045 were clearly more effective than in the rat. Also, the arachidonic acid pathway in the guinea-pig is responsible for greater than half of the CIPA produced by collagen whereas in the rat this pathway is less important. That EP045 produced the greatest inhibition of CIPA in the guinea-pig suggests that in the presence of the enzyme inhibitors, stimulation of the TXA_2 receptor still occurs. In conclusion, these results suggest that potential anti-thrombotic drugs must be studied in a number of different species until it is clear which animal species will predict clinical activity.

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MEGAKARYOCYTES IN TISSUE CULTURE: PROSTAGLANDIN BIOSYNTHESIS AND EFFECTS OF PROSTAGLANDINS ON CYCLIC AMP PRODUCTION

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Arachidonate metabolites modulate platelet function and thus are major determinants of the patho-physiological reactions in which platelets are involved (Marcus, 1978). In assessing the effects of drugs on platelet function in vivo and ex vivo, it is important to consider possible effects on megakaryocytes which impart many structural and functional components to platelets (Fedorko, 1978). Investigations of megakaryocyte function in vitro are hindered by difficulties in isolation and maintenance of megakaryocytes in tissue culture. These problems may be overcome by the use of an eternal line of rat bone marrow - derived promegakaryoblasts (RPM Cells) capable of maturing in culture to megakaryocytes (Weinstein et al, 1981). In the present study we examined the profile of RPM cell PG biosynthesis and the effects of exogenous PGs on RPM cell cyclic AMP production.

Mature RPM cells were grown in suspension culture at 37°C under 95% air/5% CO_2 in Dulbeccos modified Eagle's medium (DME) supplemented with foetal calf serum (10% v/v), benzyl penicillin (100 iu/ml) and streptomycin (100 µg/ml). Cells were harvested by centrifugation and resuspended in Hepes buffered DME, pH7.4. For measurement of PG biosynthesis, RPM cells ($0.5 - 1 \times 10^7$ cells/ml) were incubated (120 min:37°C) with [^{3}H]-arachidonate (2.5 µCi; 13.3 nM). Control samples consisting of [^{3}H]-arachidonate in Hepes-DME were processed in parallel. The cell-free supernatant was acidified, extracted with cyclohexane:ethylacetate (1:1 v/v) and the PGs separated by thin layer chromatography (ethylacetate: acetone:acetic acid; 90:10:1 v/v/v) and quantified by liquid scintillation counting. For cyclic AMP production, RPM cells were incubated (1 min : 37°C) with PGD_2, PGE_1 or PGI_2. Reactions were terminated by addition of ethanol and the cyclic AMP content measured by competitive protein binding assay.

Following incubation with [^{3}H]-arachidonate, RPM cells produced significant amounts of TxB_2, PGE_2, PGD_2, $PGF_{2\alpha}$ and 6 Keto-$PGF_{1\alpha}$. The percentages of total [^{3}H]-arachidonate metabolites that co-chromatographed with authentic standards were:- PGE_2 (9.48 ± 0.16); TxB_2 (8.34 ± 0.36); PGD_2 (7.09 ± 0.5); $PGF_{2\alpha}$ (4.25 ± 0.61) and 6 Keto-$PGF_{1\alpha}$ (1.73 ± 0.16) (mean ± S.E., n = 12). RPM cell cAMP production was stimulated (up to 10-fold) in a concentration-dependent manner by PGE_1 (0.1-10 µM) and PGI_2 (0.1-10 µM) but not by PGD_2 ($\leqslant$10 µM). These results indicate that RPM cells can synthesise an array of PGs and that the major products are similar to those generated by rat platelets and by rat bone marrow cells (Demers et al, 1980). Moreover, only PGE_1 and PGI_2, which stimulate rat platelet cyclic AMP production, are capable of stimulating RPM cell cyclic AMP biosynthesis.

We suggest that RPM cells may provide a useful model system in which the effects of drugs on megakaryocyte function can be assessed.

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ANTIARRHYTHMIC EFFECT OF METOPROLOL RELATED TO THE TIME OF ADMINISTRATION

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There is considerable evidence that catecholamines are involved in the genesis of ventricular arrhythmias resulting from acute myocardial ischaemia (Fitzgerald, 1982) and clinical trials have shown that metoprolol reduces mortality if given soon after the onset of acute myocardial infarction (Hjalmarson et al., 1981). This present study was carried out to determine the relationship between time of administration and the effectiveness of metoprolol on the early and late phase of ventricular arrhythmias resulting from acute myocardial ischaemia.

Male rats, anaesthetised with sodium pentobarbitone (60 mg/kg i.p.) were prepared for coronary artery ligation using the technique described by Clark et al. (1980). Metoprolol (2 mg/kg i.v.) was administered at various times prior to, and following ligation. The effects on the arrhythmias occurring within the 0-0.5h and 0.5-4h periods are summarised in Table 1.

Table 1. The number of ventricular ectopic beats (VEB) and percentage incidence of ventricular tachycardia (VT) and ventricular fibrillation (VF) during acute myocardial ischaemia.

Treatment	None	Metoprolol (2 mg/kg i.v.) given at:-					
		-15	+2	+5	+10	+30	+120 min
Early phase (0-0.5h)							
n	70	28	11	10	10	9	10
VEB	1131 (105)	802 (135)	1081 (287)	1246 (413)	1149 (500)	1191 (537)	1304 (283)
VF	50	18**	9*	50	45	55	60
Late phase (0.5-4h)							
n	39	19	-	-	-	9	9
VEB	565 (186)	28 (9)*	-	-	-	7 (2)*	111 (58)*
VT	31	0	-	-	-	0*	0*

Values are means ± s.e.m. (in parentheses). *P<0.05; **P<0.01. Drug treated groups were compared with a similar number of controls performed at the same times.

Pretreatment with metoprolol, and intervention with metoprolol within a very short time (2 min) following the onset of ischaemia, caused a significant reduction in the incidence of ventricular fibrillation during the 0-0.5h period. There was no effect on VEB's or VT. Intervention after the start of arrhythmic activity (i.e. 5 min or later) did not reduce the incidence or severity of the early arrhythmias. However, the number of ventricular ectopic beats and the incidence of ventricular tachycardia occurring during the late (0.5-4h) period were markedly reduced by both pretreatment and late intervention with metoprolol.

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EFFECTS OF PROLONGED INHIBITORY NERVE STIMULATION ON DRUG-INDUCED RELAXATIONS OF THE MOUSE ANOCOCCYGEUS MUSCLE

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Field stimulation of the mouse anococcygeus muscle causes a biphasic non-adrenergic, non-cholinergic relaxation (Gibson, 1983). One procedure which was found to differentiate between the two phases was a prolonged train of inhibitory nerve stimulation (10 Hz ; 10 min). Following such a train the first phase of nerve-induced relaxation was little affected but the second phase was greatly reduced. It was therefore of interest to determine the effects of prolonged inhibitory nerve stimulation on relaxations of the mouse anococcygeus muscle in response to some drugs.

Male mice anococcygeus muscles were dissected and set up for recording isometric tension responses as described previously (Gibson & Wedmore, 1981). Field stimulation was applied by two parallel platinum electrodes running down either side of the tissue (1 msec pulse width ; supramaximal voltage). In order to prevent effects of sympathetic nerve stimulation the muscles were preincubated with guanethidine (30 μM ; 15 min) and the Krebs solution contained phentolamine (1 μM). In addition, when responses to adenosine 5'-triphosphate (ATP) were to be studied the tissue was also preincubated with indomethacin (2.8 μM ; 1 hour). Muscle tone was raised with carbachol (50 μM).

Carbachol-induced tone of the mouse anococcygeus muscle was reduced by the following drugs : vasoactive intestinal polypeptide (VIP, 0.02-2 μM) ; sodium nitroprusside (10-400 nM) ; ATP (0.6-10 mM) ; 3-isobutyl-1-methylxanthine (IBMX, 2-100 μM) ; and papaverine (1-100 μM). In addition, extract of bovine retractor penis (Gillespie, Hunter & Martin, 1981, 50 and 100 μl/ml) caused relaxation. However, nifedipine did not relax carbachol-induced tone (up to 400 nM). The effect of prolonged inhibitory nerve stimulation was determined on concentrations of the above compounds which produced about a 50% reduction of carbachol-induced tone. Following a 10 min train of inhibitory nerve stimulation (10 Hz) the percentage change in the magnitude of the relaxations produced were as follows : 1 μM VIP (-88 ± 3%, n = 6) ; 100 nM nitroprusside (-66 ± 4%, n = 6) ; 4mM ATP (+2 ± 4%, n = 12) ; 20 μM IBMX (-7 ± 5%, n = 8) ; 20 μM papaverine (-8 ± 2%, n = 6) ; and 100 μl/ml bovine retractor penis extract (-16 ± 4%, n = 7). Thus prolonged inhibitory nerve stimulation greatly reduced relaxations to VIP and nitroprusside but had little effect on responses to ATP, IBMX, papaverine, or bovine retractor penis extract.

These results suggest that, 1) carbachol produces contraction of the mouse anococcygeus muscle via a nifedipine-resistant, tonic (T) mechanism (Golenhofen, 1981), and 2) the mechanism by which VIP and nitroprusside reduce carbachol-induced tone may be similar to that of the second phase of non-adrenergic, non-cholinergic inhibitory nerve stimulation.

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THE EFFECT OF ADENOSINE TRIPHOSPHATE ON TETRODOTOXIN AXONAL BLOCKADE

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It has been described that the effect of adenosine triphosphate (ATP), related nucleotides and adenosine on the release of the transmitter at the neuromuscular junction depends on previous neuromuscular depression (Ribeiro,1981). The present work was undertaken to investigate the effect of ATP on the axonal blockade induced by tetrodotoxin (TTX).

The experiments were carried out at room temperature (22 - 25^{o}C) on the partially desheathed frog sciatic nerve trunk. The preparations were arranged so that the solutions containing the drugs could be applied to the desheathed part of the trunk. The nerve was stimulated supramaximally with rectangular pulses of 0.01 ms duration applied once every 5 s. Throughout the experiments compound action potentials were recorded photographically using conventional amplification and recording techniques. The bathing solution contained (mM): NaCl 117; KCl 2.5; NaH_2PO_4 1; Na_2HPO_4 1; $MgCl_2$ 1.2; $CaCl_2$ 1.8 (pH 7.0).

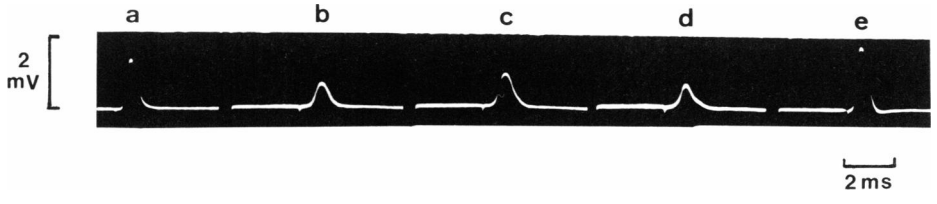

Figure 1 Effect of ATP (7.5mM) on the amplitude and duration of the frog sciatic nerve compound action potential. a) Pre-control; b) after 5 min in 60 nM TTX; c) after 5 min in 60 nM TTX + 7.5 mM ATP; d) after 5 min of returning to 60 nM TTX; e) Post-control recorded 10 min after drug-free solution. Each trace being of three consecutive superimposed compound action potentials.

Figure 1 illustrates the increase caused by ATP (7.5 mM) on the amplitude and duration of the compound action potential in the presence of TTX (60 nM). ATP increased by 33% the amplitude of the action potential. Dose-dependent increases in the amplitude of the action potential were observed in six separate experiments in which ATP (0.5 - 7.5 mM) was applied in the presence of TTX (15 - 80 nM). The effect was more pronounced where the blockade was more intense. For example, ATP (5 mM) increased by 80 - 100% the amplitude of a compound action potential which has previously been reduced by TTX to 0,1 - 0.2 times its initial (pre-control) value, whereas, it increased it by 25 - 35% when the reduction in TTX was 0.4-0.5 times the pre-control action potential amplitude.

These results indicate that ATP can partially antagonize the effects of TTX on nerve conduction.

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A COMPARISON BETWEEN NEUROMUSCULAR EFFECTS OF TETRACYCLINE AND POLYMYXIN B

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In a previous report to this society it was shown that polymyxin B, in low concentrations, may facilitate neuromuscular transmission while it greatly reduces the contracture responses produced by acetylcholine (ACh) in the isolated chick biventer cervicis nerve-muscle preparation.

In this communication, the pharmacological actions and interactions of another class of antibiotic agent, tetracycline, were studied and the results were compared with those previously obtained by polymyxin B in the same preparation and in the rat phrenic-hemidiaphragm preparation.

The preparation was set up in an organ bath containing Krebs-Henseleit solution maintained at 38°C and bubbled with 95% O_2 and 5% CO_2. Twitch contractions elicited at 0.2 Hz with 5V and 0.5 ms pulse duration and contractures produced by ACh and tetraethylammonium (TEA) were recorded using a semi-isometric tension recording force transducer and a Washington pen recorder.

Unlike polymyxin B, tetracycline (16 μM) had only a slight effect on the amplitudes of the twitch contractions produced by repetitive nerve stimulation at 0.2 Hz (reduced by 7% ± 0.8, n=6). However, both tetracycline and polymyxin B (1.31 μM) reduced the amplitudes of the contractures produced by ACh (0.55-11.0 mM) and TEA (2.4-12.0 mM). Unlike polymyxin B, tetracycline shifted the concentration-response curve, produced by ACh in the control Krebs solution, to the right in a competitive manner. The mean (± SEM) ED50s for the contractures produced by ACh in the control Krebs solution and in tetracycline (16 μM) were 1.2±0.05 mM and 4.3±0.06 mM, n=6, respectively. Higher concentrations of both polymyxin B and tetracycline produced a neuromuscular blockade which was not reversed by neostigmine and only partially reversed by calcium. Similar results for the actions of polymyxin and tetracycline were obtained in the isolated rat phrenic-hemidiaphragm preparation.

The effects of polymyxin B and tetracycline on vertebrate neuromuscular transmission have been studied by many workers (Van Nyhuis, Miller & Fogdall, 1976; Wright & Collier, 1976; Lee, de Silva & Katz, 1978; Singh, Marshall & Harvey, 1978). These authors have suggested that different groups of antibiotics act by different mechanisms and that both pre- and postsynaptic actions may be observed at the vertebrate neuromuscular junction (for reviews see Pittinger & Adamson, 1972; Singh, Marshall & Harvey, 1980).

In conclusion, the present results show that the neuromuscular actions of polymyxin B were different from those produced by tetracycline, indicating that the two classes of antibiotics may act by different mechanisms at the vertebrate neuromuscular junction.

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AXONAL TRANSPORT, NERVE CONDUCTION AND SUGAR CONTENT IN ACUTE EXPERIMENTAL DIABETES IN RATS AND THE EFFECTS OF INSULIN TREATMENT

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Acute streptozotocin diabetes in rats causes impaired orthograde axonal transport and a concomitant reduction in motor nerve conduction velocity (MNCV) in the sciatic nerve (Mayer & Tomlinson, 1982a). Both defects are prevented by administration of an inhibitor of aldose reductase (Tomlinson, Holmes & Mayer, 1982), an effect which may be related more to normalisation of nerve *myo*-inositol content than to prevention of sorbitol formation by the enzyme (Mayer & Tomlinson, 1982b). The present study was designed to determine whether control of diabetes with insulin affected the development of these neurological defects. Three groups of rats (male Wistar 300-310 g) were studied over three weeks. Two groups were made diabetic (streptozotocin 50 mg/kg i.p.) and one of these received twice-daily insulin starting 3 days after streptozotocin. At 0830 hrs each morning blood glucose was measured and each rat was given 2 to 3 U/100 g (s.c.) Semitard insulin MC (Novo) relative to glycaemia. At 1700 hrs Monotard insulin MC (Novo) was given at the same dose for each rat. MNCV (sciatic nerve) was measured the day before streptozotocin and again 21 days later in diabetic rats and twice 21 days apart in controls. On the last day the accumulation of choline acetyltransferase activity, proximal to a 24 h constriction on the left sciatic nerve, was measured as an index of orthograde axonal transport. At sacrifice blood glucose and sciatic nerve sorbitol and nerve *myo*-inositol contents were measured. All procedures are described elsewhere (Tomlinson et al., 1982). The results are shown in the Table. The untreated diabetic rats showed reduced MNCV and ChAT accumulation together with accumulated sorbitol and lowered *myo*-inositol in the sciatic nerve. Insulin treatment controlled the hyperglycaemia (mean daily blood glucose (days 7 to 21)= 10.0 ±0.7 (SEM) m mol/l) and reversed all the neurological defects. Other work has shown that, in uncontrolled diabetes, aldose reductase inhibition normalises nerve sorbitol and *myo*-inositol and that oral *myo*-inositol restores nerve *myo*-inositol without affecting nerve sorbitol. Both treatments prevented the development of the defects of MNCV and ChAT accumulation (Mayer & Tomlinson, 1982b). The present findings therefore lend support to the implication of reduced nerve *myo*-inositol content as a possible cause of certain neurological defects in acute experimental diabetes.

Mean values (±SEM) after 3 weeks' diabetes (Number of rats in brackets)

Treatment	MNCV (m/sec)	ChAT accumulation (n mol ACh/h/nerve)	Sorbitol (n mol/mg nerve)	*myo*-Inositol	Blood glucose (m mol/l)
Controls (15) (non-diabetic)	52.3±1.5 *	5.1±0.4 *	0.08±0.01	2.30±0.10 ***	6.3±0.2
Diabetic (9) (untreated)	42.7±2.0 **	2.7±0.5 *	1.56±0.22	1.47±0.10 **	22.5±1.4
Diabetic (5) (daily Insulin)	54.0±2.4	5.1±0.2	0.06±0.02	2.62±0.29	9.0±1.6

*p < 0.01, **p < 0.01, ***p < 0.05, by unpaired t-tests.

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PARTIAL PURIFICATION OF A TOXIN PRESENT IN SALINE EXTRACTS OF THE SEA ANEMONE TEALIA FELINA

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Aldeen et al (1981) described a toxic compound(s) present in saline extracts of the sea anemone Tealia felina.Partial purification by gel chromatography with A50m agarose followed by sephadex G100 yielded a fraction (Extract II) which produced a slow contraction of the guinea pig ileum and blocked contractions of the ileum produced by either histamine or acetylcholine.It also haemolysed human erythrocytes.
Using the histaminolytic action on the guinea pig ileum to bioassay the extract we continued purification.Activity was measured in active units (A.U.).A solution containing 0.05 A.U./ml inhibited histamine induced contractions by 50% (Aldeen et al,1981).Protein concentration was measured by the method of Waddell (1956).

Extract II was desalted and concentrated and the solution containing 118 A.U./mg of protein was applied to a column of Amicon Blue B dyematrex gel (1 x 30 cm, flow rate 10ml/h).After washing with 43mM NaCl to remove unbound material the column was eluted with a saline gradient,10 ml fractions were collected.Activity appeared in fraction 9 (yield 15%,292 A.U./mg protein) and fraction 10 (yield 32%, 657 A.U./mg protein) corresponding to NaCl concentrations of 267 and 444mM respectively.

Fraction 10 (Extract III) was desalted and concentrated and then applied to a CMC cation exchange column (1 x 30 cm,flow rate 10 ml/h).The starting buffer was 10 mM ammonium acetate,pH5.The column was eluted with a 0-2M NaCl gradient in 10mM ammonium acetate and 5.5 ml fractions were collected.Histaminolytic activity was present in fractions 16 and 17 (Extract IV) whereas the peak protein concentration (monitored by UV absorption at 280nM)appeared in fractions 13 and 14.The yield was 30%.
We are studying Extract IV for haemolytic action and continuing purification by preparitive electrophoresis.

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AN ANTIARRHYTHMIC ACTION OF ADENOSINE IN GUINEA-PIG ISOLATED ATRIA

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A hypokalaemic bathing medium (BM) produces transient afterdepolarizations and aftercontractions in cardiac muscle preparations. An increase in intracellular calcium ion concentration ($[Ca^{++}]_i$) is suggested to be responsible for the genesis of such arrhythmias (Eisner & Lederer, 1979). They are enhanced under conditions where the $[Ca^{++}]_i$ is increased (Wit et al, 1980). In this investigation the effect of adenosine (ADO) on the arrhythmias produced by hypokalaemia, together with ß-adrenoceptor stimulation, with isoprenaline (ISO) and theophylline (THEO) induced cAMP phosphodiesterase inhibition was examined in guinea-pig atria.

Isolated paced (2 Hz, 5 ms) left atria were set up in an organ bath in a hypokalaemic (potassium concentration 1.6 mM) BM. After 30 min, during which time bigeminal rhythms and bursts of tachyarrhythmias were observed, THEO, $3x10^{-4}$M, and ISO, $1x10^{-5}$M, were added to the BM. The atria then beat spontaneously and developed tachyarrhythmias which, in control experiments, persisted for at least 30 min. After the development of arrhythmias, ADO was added cumulatively to the BM. At $1.4x10^{-3}$M, arrhythmia was inhibited in 56 % of the atria. Inhibition of ADO tissue uptake by $1x10^{-7}$M 6-(p-nitrobenzyl)-thioinosine (NBMPR) resulted in ADO being significantly more effective, inhibition being achieved in all atria at $1.1\pm0.5x10^{-4}$M ADO. In the presence of NBMPR and adenosine deaminase ($2x10^{-1}$ u/ml), ADO $1.4x10^{-3}$M did not inhibit the arrhythmias.

In further experiments, the action potential (AP) of paced (1 Hz, 1 ms) left atrial strip cells was recorded using standard micro-electrode techniques (Table 1). ADO reduced the AP duration of atrial cells in Krebs-bicarbonate solution at 10% (APD_{10}) and 90% (APD_{90}) repolarisation (Table 1, B). NBMPR enhanced this effect (Table 1, C). After short periods of exposure of the strips to ISO and THEO in hypokalaemic BM, afterdepolarizations were observed arising from high and low levels of membrane potential. After more prolonged periods of exposure, triggered tachyarrhythmias were recorded. The AP arising from a resting potential (RP), not significantly different from that of control cells (Table 1, D). After rhythm restoration by ADO and NBMPR, the APD_{90} and overshoot (OV) were significantly reduced (Table 1, E).

Table 1 Action potential parameters of guinea-pig atrial cells

	BM	APD_{10}(ms)	APD_{90}(ms)	RP(mV)	OV(mV)	dV/dt_{max} phase 0 (V/s)
A	Krebs-bicarbonate	9.3±0.5	71.5±7.1	-74.1±0.9	17.0±0.9	144.1± 5.8
B	A + ADO $3x10^{-4}$M	3.8±0.3*	48.5±2.8*	-76.7±1.0	15.9±1.0	137.9± 7.7
C	B + NBMPR $1x10^{-7}$M	2.5±0.2+*	24.8±1.6+*	-79.0±1.0	11.4±0.7	153.5± 8.8
D	Hypokalaemia + ISO $1x10^{-5}$M, THEO $3x10^{-4}$M	4.5±0.6	75.9±2.9	-79.5±2.6	20.8±1.5	146.1±22.1
E	D + ADO $3x10^{-4}$M, NBMPR $1x10^{-7}$M	3.9±0.4	47.1±3.1x	-87.1±3.3	10.5±2.3x	149.6± 9.6

*+x significantly different from A, B and D respectively ($p<0.01$), D recorded from spontaneously beating tachyarrhythmic atria.

In conclusion, ADO has been shown to be antiarrhythmic via an interaction with its extracellular receptor. This activity is presumably due to the observed shortening of the APD by ADO. This is likely as the latter effect is associated with a reduction in $[Ca^{++}]_i$, an action that abolishes afterdepolarizations (Wit et al, 1980).

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FURTHER OBSERVATIONS ON ADENOSINE DEPENDENCE IN GUINEA-PIG ILEUM IN VITRO

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Incubation of guinea-pig isolated ileum with adenosine induces in the final cholinergic motoneurone of the myenteric plexus a dependence manifested as a contracture of the longitudinal muscle, after removal of adenosine or its antagonism with 8-phenyltheophylline (8-PT) (Collier & Tucker, 1983). We have extended this work using the same method. After preincubation for 16-21 h at 18-22°C in Krebs solution containing 70 µM hexamethonium with or without inducing drug, preparations were set up at 37°C in fluid of the same composition for recording contracture of the longitudinal muscle. After responses to electrical pulses and to acetylcholine (ACh) had been recorded, the preparation was challenged with antagonist and the response expressed as a percentage of the maximal response to ACh.

Dependence induction by adenosine and precipitation of withdrawal by 8-PT were dose-related. Thus, in comparable experiments, challenge with 10 µM 8-PT of ileum preincubated in 1 µM adenosine gave a mean response of 24.6 ± 4.6 s.e.m. % (n=7), which was significantly less than the response obtained after preincubation in 4 µM adenosine (67.1 ± 6.4 %; n=10; $P<0.001$). Again, withdrawal precipitated with 2.5 µM 8-PT was 40.3 ± 7.4 % (n=10), which was significantly less than that precipitated with 10 µM 8-PT (67.1 ± 6.4 %; n=10; $P<0.02$).

Like adenosine, but more potently, 2-chloroadenosine (2-CA) induced dependence; and, like 8-PT, but less potently, caffeine precipitated abstinence. Thus, preincubation with 60 nM 2-CA yielded a withdrawal contracture to 10 µM 8-PT of 31.5 ± 4.8 % (n=11). Again, after preincubation in 4 µM adenosine, 1 mM caffeine elicited a contracture of 46.9 ± 7.3 % (n=7), which was significantly more than control (24.4 ± 3.1 %; n=7; $P<0.02$).

Neither naloxone (0.1 µM) nor yohimbine (1 µM) precipitated a withdrawal contracture in ilea incubated with adenosine and responsive to 8-PT. Normorphine (1 µM) or clonidine (0.1 µM), however, suppressed the withdrawal contracture to 8-PT in adenosine-dependent ilea. The recognition site in adenosine dependence is therefore distinct from those in opiate or clonidine dependence (Collier et al, 1981a,b), but the final path of the withdrawal effects is common. Since adenosine occurs in brain (Stone, 1981) and caffeine precipitated a withdrawal contracture of adenosine-dependent ileum, precipitation of withdrawal in brain neurones that have become adenosine-dependent might contribute to caffeine arousal in vivo.

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RECEPTOR BINDING CHARACTERISTICS OF MUSCARINIC ANTAGONISTS IN BOVINE TISSUES

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The selective effects of a number of muscarinic antagonists on gastric acid secretion have been investigated in vivo (Heathcote and Parry, 1980). However only the muscarinic receptor binding characteristics of pirenzepine have been further investigated using receptor-ligand binding techniques. Therefore the interaction of a range of antagonists with muscarinic receptors has been compared in two bovine tissues; gastric smooth muscle from the abomasum and superior cervical ganglia. Receptor membrane fragments were incubated at 30°C, at 1 mg protein/ml in HEPES buffer (pH 7.5) for a period of 20 min, at which time binding was at equilibrium. The reaction was terminated by rapid filtration (Whatman GF/F filters). Concentration-occupancy curves for the antagonists were derived indirectly from competition experiments against 0.5 nM [3H]-N-methylscopolamine. The results are shown in Table 1.

Table 1 Specific binding of muscarinic antagonists to homogenates of bovine gastric smooth muscle (GSM) and superior cervical ganglia (SCG)

Drug	GSM		SCG		IC50 RATIO
	IC50 (nM)	nH	IC50 (nM)	nH	GSM/SCG
Atropine	4.3	1.0	2.9	1.0	1.5
N-methylscopolamine	0.31	1.0	0.46	0.97	0.67
Oxyphencyclimine	3.3	0.99	4.1	0.96[b]	0.80
Pirenzepine	370	0.93	27[a]	0.66[b]	14
Glycopyrronium	1.3	0.90	1.0	0.94	1.3
Isopropamide	1.8	0.93	2.9	0.98	0.62
Poldine	3.0	0.91	6.0	0.89	0.50
Propantheline	0.59	1.0	0.40	0.88	1.5

IC50 corrected for radioligand-induced shift; nH = the Hill coefficient;
a $P < 0.01$, unpaired Student's 't' test comparing IC50's in both tissues;
b $P < 0.01$, nH significantly different from unity; results are the mean of three experiments.

With the exception of pirenzepine each drug had binding properties consistent with classical muscarinic antagonists. In both tissues they bound to a homogeneous population of receptors (nH=1) with IC50 values differing by no more than a factor of 2.0. However, pirenzepine had 14 times the affinity for receptors in ganglia than in smooth muscle suggesting that different affinities of pirenzepine for muscarinic receptors may contribute to the selective antisecretory properties observed in vivo. The Hill coefficient of 0.66 in ganglia suggests that pirenzepine recognised more than one receptor population. The failure of these in vitro studies to confirm the in vivo selectivity of oxyphencyclimine suggests a different but unknown mechanism of action.

It is concluded therefore, with this one exception, that the affinity measurements of the drugs in these tissues correlate well with drug selectivity seen in vivo.

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MODIFICATION OF OESTROGEN INDUCED RESPONSES IN THE RAT UTERUS BY HISTAMINE ANTAGONISTS

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Responses of the rat uterus to oestrogens within 6h of treatment include an increase in blood flow and an increase in uterine weight, part of the latter is due to water imbibition (Spaziani, 1975). Treatment with the anti-oestrogen, tamoxifen, inhibited the oestrogen induced hyperaemia and weight increase seen in the rat uterus suggesting that receptor binding is a pre-requisite for these events to occur (Majid and Senior, 1982). Local hormones have been implicated in the oestrogen induced response in the rat uterus (Phaily & Senior, 1978) and the purpose of this work is to investigate the part played by histamine in these early uterotrophic events.

Mature rats (CD-derived) were bilaterally ovariectomized and allowed at least 14 days to recover before being used for further experiment. Blood flow was measured in the anaesthetized rat by the microsphere (15μm NEN-Trac) technique (Phaily & Senior, 1978). Uterine blood flows are expressed using tissue wet weights, both uterine wet and dry weights were recorded.

The H_1-antagonist, mepyramine (5mg kg^{-1} i.p.) given 30 min before oestradiol (0.5μg kg^{-1} i.v.) significantly reduced ($P < 0.01$) the oestrogen induced uterine wet and dry weight increase 3h after the oestrogen injection but had no effect on the hyperaemia. At 6h after the oestrogen treatment (mepyramine dose was repeated 3h after oestrogen) only the dry weight was reduced by the presence of the H_1-antagonist ($P < 0.05$).

Ranitidine (1mg kg^{-1} i.v.) given alone reduced uterine dry weight ($P < 0.05$) within 3h treatment but this response had disappeared by 6h (ranitidine dose was repeated at 3h). Like mepyramine, ranitidine inhibited uterine wet and dry weight increases 3h after oestradiol treatment ($P < 0.01$) but ranitidine also increased the oestrogen stimulated blood flow from 735 ±66 to 1183 ±117 ml min^{-1} $100g^{-1}$ ($P < 0.01$). The 6h oestrogen induced uterotrophic events were not modified by the presence of ranitidine. A combination of ranitidine and mepyramine treatment greatly reduced ($P < 0.001$) the oestrogen stimulated increase in wet weight 3h after the oestrogen injection, the dry weight was also significantly lower ($P < 0.01$); the blood flow was unaffected.

Both the histamine H_1 and H_2 receptors appear to be involved in the very early (3h) uterine weight responses to oestrogen. The weight increases 6h after oestrogen do not involve histamine receptor occupancy. Blood flow responses to oestrogen at 3h may involve histamine activity as ranitidine increased the uterine hyperaemia but the presence of mepyramine inhibited this effect.

We thank Glaxo Group Research for the gift of ranitidine.

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PHENYLEPHRINE-INDUCED ACTIVITY IN MICE AS A MODEL OF α_1-ADRENOCEPTOR FUNCTION: STUDIES OF VARIOUS ANTIDEPRESSANT TREATMENTS

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Repeated administration of some antidepressant drugs or electroconvulsive shock (ECS) decreases both β-adrenoceptor number (Wolfe et al, 1978; Bergstrom & Kellar, 1979) and presynaptic α_2-adrenoceptor function (Sugrue, 1981; Przegalinski et al, 1981; Heal et al, 1981). However, there have been few studies of the effects of these treatments on α_1-adrenoceptor-mediated behavioural changes; probably because α_1-agonists do not penetrate the blood-brain barrier. This difficulty has been circumvented by using an intracerebroventricular injection technique, and the behavioural effects of the α_1-agonist phenylephrine are now reported. Furthermore, the effects of some antidepressants and repeated ECS on this model have also been studied.

All experiments were performed on adult male C57/Bl/6Ola mice (c. 30 g). The activity of groups of 2 mice were recorded using LKB Animex activity meters (sensitivity and tuning 30 μA). Phenylephrine (dissolved in 2 μl saline) was injected intracerebroventricularly into conscious mice using a simple stereotaxic device. The head was located by lining the eyes against predetermined reference marks and the skull was then pierced by passing a fixed length needle through a guide. Histological examination after dye injection showed a success rate >80%. Other drugs were given by intraperitoneal injection.

Following injection of phenylephrine (10-100 μg icv) mice displayed a dose-dependent increase in locomotor activity accompanied by various characteristic behavioural changes. The mice walked with a pronounced rolling gait which was generally performed with the body hunched, head held down and stomach held clear of the cage floor. The behavioural changes were tremor, marked piloerection, stretching of the hindquarters, proptosis, flattened ears and Straub tail. During periods of maximal activity, mice often repeatedly jumped around the cage. These changes appeared to be specifically mediated by central α_1-adrenoceptors because the activity produced by phenylephrine (25 or 50 μg icv) was markedly reduced by pretreatment with prazosin (3 mg/kg) but was unaltered by administration of either yohimbine (1 mg/kg) or propranolol (2.5 mg/kg). When mice were tested 90 min after the acute administration of various antidepressant drugs, the behavioural activity produced by phenylephrine (25 μg icv) was markedly reduced by mianserin (5 mg/kg), but unaltered by either desmethylimipramine (DMI; 5 mg/kg) or amitriptyline (5 mg/kg). Mice were also injected with DMI (5 mg/kg) or saline twice daily for 14 days. On day 17, 60 h after the final treatment, when both groups of mice were injected with phenylephrine (25 μg icv), there were no differences between their behavioural responses. In further experiments, mice were lightly anaesthetised and given a single ECS (110 V, 1 s) once daily for 10 days, while controls received anaesthetic alone. When both groups of mice were tested 24 h after the final treatment, the behavioural responses of the 2 groups to phenylephrine (25 μg icv) were identical. Together these data suggest that when given acutely, mianserin, unlike DMI or amitriptyline, is a potent antagonist of α_1-adrenoceptor-mediated behavioural changes. In addition, ECS or DMI given repeatedly, using protocols which decrease presynaptic α_2-adrenoceptor function in this strain of mice (Heal et al, 1981; Heal & Green, in preparation), do not alter the α_1-adrenoceptor-mediated behavioural activity.

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PARACETAMOL HEPATOTOXICITY: EVIDENCE FOR THE INVOLVEMENT OF N-ACETYL-P-BENZOQUINONEIMINE

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The hepatotoxicity of paracetamol is mediated by a reactive intermediate formed by a cytochrome P-450 mixed function oxidase in the liver (Mitchell et al, 1973). The identity of the reactive intermediate and its mechanism of toxicity are unknown, although the toxicity correlates closely with its degree of covalent binding to hepatic macromolecules (Jollow et al, 1973). Of the candidates proposed as the toxic species N-acetyl-p-benzoquinoneimine (NABQI) or a semiquinone radical are the most likely. We present data that supports NABQI as the reactive intermediate and provide evidence against the involvement of a radical intermediate in the cytochrome P-450 mediated hepatotoxicity of paracetamol.

Chemically synthesised ^{14}C-NABQI (Huggett & Blair, 1983a) was reacted with the radical scavengers butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA). BHA reacted with ^{14}C-NABQI by a redox mechanism in which ^{14}C-NABQI was completely reduced to ^{14}C-paracetamol. With BHT only a small amount of ^{14}C-NABQI was consumed.

The effect of these two compounds upon the in vitro covalent binding of ^{14}C-paracetamol to mouse hepatic microsomes was investigated. The results are summarised in Table 1. BHA completely prevented the NADPH-dependent binding while BHT only inhibited total binding by 18%.

The results indicate that BHA can function as a reducing agent as well as a radical scavenger. NABQI, which has previously been shown to be a potent oxidising agent (Huggett & Blair, 1982), is reduced to paracetamol by BHA. The effect of the radical scavengers upon the covalent binding of paracetamol indicates that the hepatic microsomal NADPH-dependent activation of paracetamol does not result in the production of a radical intermediate. The ability of BHA but not BHT to inhibit the covalent binding supports the identity of NABQI as the reactive intermediate. This study confirms the proposal that reducing agents, such as BHA, may be of value in preventing paracetamol-induced hepatotoxicity in vivo (Huggett & Blair, 1983b).

Table 1 Effect of radical scavengers on in vitro covalent binding of paracetamol (2 mM)

Radical Scavenger (2 mM)		Covalent Binding (n = 2) nmoles bound/mg protein/10 min
None	(+ NADPH)	5.4
None	(- NADPH)	1.2
BHT	(+ NADPH)	4.4
BHA	(+ NADPH)	0.9

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HISTAMINE RELEASE FROM MECHANICALLY AND ENZYMATICALLY DISPERSED HUMAN LUNG MAST CELLS: INHIBITION BY SALBUTAMOL AND CROMOGLYCATE

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Experiments with human lung fragments have suggested that the beta-adrenoceptor agonist, salbutamol, is a potent inhibitor of IgE-dependent histamine release, whereas sodium cromoglycate is only weakly active (Church & Young, 1983). However, results using this model are quantitatively inconsistent and give only a gross indication of drug efficacy (Young & Church, 1983). We describe the activity of these drugs in dispersed human lung cells prepared by mechanical and enzymatic methods.

Mechanically dispersed cells were prepared from fresh human lung tissue (Church, Pao & Holgate, 1982). Enzymatically dispersed cells were prepared by digestion of lung fragments with pronase (2mg/ml) and chymopapain (0.5mg/ml) for 30 minutes at 37°C in a HEPES-Tyrode buffer at pH 7.4. Free cells were separated by filtration, washed and resuspended in HEPES-Tyrode buffer with 0.03% human serum albumin. Cell aliquots, containing 1-5% mast cells were incubated with salbutamol or sodium cromoglycate for 5 minutes prior to challenge with goat anti-human IgE. After 15 minutes' incubation at 37 °C reactions were terminated by centrifugation at 4°C and net histamine release measured by spectrofluorimetry (Church et al, 1982).

Incubation with 1/10, 1/100 and 1/300 dilutions of anti-IgE induced a net histamine release of 21.8±3.8, 17.5±6.5 and 14.0±5.1% (mean±s.e.mean, n=4), from mechanically dispersed mast cells and 36.2±8.9, 24.1±8.2 and 18.8±9.290 from enzymatically dispersed cells. These differences between cell preparations were highly significant ($p<0.001$). Spontaneous release in the absence of challenge was not different, being 12.7±1.9 and 14.2±1.1% respectively. In both mast cell preparations, salbutamol 0.001-10μM produced similar dose-related inhibitions of histamine release. However, the degree of inhibition caused by salbutamol was inversely related to the concentration of anti-IgE used for challenge ($r= -0.98$). The maximal effective concentration of salbutamol at all anti-IgE concentrations was 1μM. This produced a mean inhibition of 17.7±2.0%, 34.8±4.7% and 51.0±7.2% in cells challenged with 1/10, 1/100 and 1/300 dilutions of anti-IgE respectively. The inhibitory effects of sodium cromoglycate (10-1000μM) were weak but again appeared inversely related to anti-IgE concentrations. At an anti-IgE concentration of 1/300, 1000μM sodium cromoglycate produced 28.7±5.7% inhibition of histamine release.

In comparison with mechanically dispersed mast cells, enzymatically dispersed cells were more responsive to immunological stimulation, but the characteristics of inhibition by salbutamol and sodium cromoglycate were similar. In both preparations the efficacy of these drugs was highly dependent on the degree of immunological stimulation.

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RECEPTORS ON THE MEMBRANES OF PASSAGED HUMAN EPIDERMAL KERATINOCYTES IN CULTURE

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Membrane receptors in passaged human epidermal keratinocytes have been investigated using both binding studies and activation of membrane-bound adenylate cyclase. Mammary skin obtained from plastic surgery and infant foreskin were used as sources for the keratinocytes. The cells were cultivated during 2-3 passages and were taken for the preparation of membranes after reaching confluency.

Using binding studies, keratinocyte membranes were found to possess ß-adrenergic receptors. From the binding of tritiated antagonists, an equilibrium dissociation constant (K_d) of 1.3 nM was found for ^{3}-H-dihydroalprenolol and a receptor density of 280 fmol/mg membrane protein. In association and dissociation kinetic experiments, the equilibrium dissociation constant, obtained from the ratio of the dissociation and association rate constants, was similar to the value determined under equilibrium conditions. Similar agreement was found also for receptor density values, suggesting that the binding to these receptors is a bimolecular reaction. From displacement studies with subtype specific antagonists, the ß-adrenergic receptors have been sub-classified as homogenous $ß_2$ receptor population.

The functional integrity of the ß-adrenergic receptors has been investigated by measuring adenylate cyclase activation by agonists binding. The isolated membranes were found to have adenylate cyclase which could be stimulated by GTP or its non-hydrolysable analogue 5'-guanylylimidodiphosphate, by NaF or by cholera toxin. Isoprenaline activated the adenylate cyclase stereospecifically (no activation by the D-isomer) and this activation could be completely reversed by the addition of ß-adrenergic antagonists such as propranolol or alprenolol. Enzyme activation as a function of agonist concentration has been measured for L-isoprenaline, L-adrenaline and L-noradrenaline. The activation constants have been calculated for each agonist (K_a, the agonist concentration required to achieve 50% activation) and were found to be very close to the values of the equilibrium dissociation constants (K_d) determined for these agonists in binding experiments (Table 1).

	L-Isoprenaline	L-Adrenaline	L-Noradrenaline
K_a (activation)	0.4 µM	1.7 µM	57.6 µM
K_d (binding)	0.6 µM	5.7 µM	70.7 µM

The close agreement between the K_a and the K_d values suggests that complete receptor occupancy is required to achieve maximal enzyme activation. In all the experiments reported above, no difference was found between cells derived from either infant or adult skin donors.

Up to now, no specific binding could be measured with either α-adrenergic (Prazosin and Yohimbine) or with histamine (Doxepin) antagonists. In the adenylate cyclase assay, no activation of the enzyme was found with histamine or with the prostaglandins $F_{2\alpha}$ and E_2. Phentolamine had no measurable effect on the activation of adenylate cyclase by noradrenaline.

We conclude from these studies that passaged human keratinocytes in culture possess functional ß-adrenergic receptors, but do not have either histamine, prostaglandin or α-adrenergic receptors.

COMPETITIVE BINDING STUDIES WITH DIPYRIDAMOLE AND α_1-ACID GLYCOPROTEIN

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Dipyridamole (DPD) is a clinically effective vasodilator and platelet anti-aggregatory agent. Its effectiveness is limited because of its marked binding affinity for plasma α_1 acid glycoprotein (AGP) (Subbarao et al (1977)).

All fluorescence measurements were made using a Baird Atomic Ratiometric spectrofluorimeter model RC200. The polarisation readings were made using the Baird polariser in this instrument. DPD is a highly fluorescent compound with two excitation maxima at 305 nm and 420 nm and an emission maximum at 490 nm. Using an excitation wavelength of 295 nm, a wavelength which will only excite the tryptophan residues on the AGP (Chignell (1970)), and an emission wavelength of 335 nm, it was found that DPD binding quenched the fluorescence of the protein, this being associated with an increase in the fluorescence of the DPD thus indicating resonance transfer of energy. Using the method of analysis suggested by Chignell et al (1970) for binding with quenching, association constants k_i = 4.07 $\pm$ 0.4 x 10^7 M^{-1} and k_2 = 5.70 $\pm$ 0.35 x 10^5 M^{-1} were obtained. The quenching effect was paralleled by a marked fluorescence polarisation at the 420 nm peak. The polarisation data was analysed using the method of Guarino et al (1973) and gave association constants k_i = 2.88 $\pm$ 0.15 x 10^7 M^{-1} and k_2 = 6.98 $\pm$ 0.3 x 10^5 M^{-1}. The data obtained suggested that one DPD molecule binds to two sites on the AGP with different association constants. Urea which causes the protein to unfold and form a random coil abolished both the quenching and the polarisation effect. The extent of quenching suggested that more than one tryptophan residue is involved in the binding. A cross linking of DPD between strands of the chain in the protein molecule is envisaged. These findings confirm and extend the observations of El-Gamel (1982) who used optical rotation measurements to study the interaction between DPD and AGP.

For competitive studies, fluorescence polarisation was used because it was specific for DPD binding. The specificity of this binding was demonstrated by the fact that RA233 (2,6-bis(diethanolamino)-4-piperidino-pyrimido-(5,4-d) pyrimidine), structurally very similar to DPD, did not quench the protein's fluorescence or show a change in polarisation on binding. We found that hydrophobic planar molecules compete most efficiently for the DPD primary binding site. Examples of competitors given in order of inhibition constants (Segal (1975)) are RA233 K_i = 1.5 x 10^{-5} M, papaverine K_i = 2.5 x 10^{-5}M, SH 1242 (5-methyl-3 piparazinyl-1-(-thiomorpholinyl) isochinolin-s-oxide) K_i = 3.1 x 10^{-5} M and propranolol K_i = 3.7 x 10^{-5}M. Compounds that did not compete include adenosine, caffeine and methotrexate.

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PAF-ACETHER INDUCED PLASMA PROTEIN EXTRAVASATION IN GUINEA-PIG SKIN IS PLATELET INDEPENDENT

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PAF-acether is a potent aggregator of guinea-pig platelets and produces platelet-dependent bronchoconstriction in this species (Vargaftig et al., 1981). Intradermal injection of synthetic PAF-acether (0.1-100 ng/site) in guinea-pigs induces plasma protein extravasation (PPE) associated with platelet accumulation (PA) at the higher doses (Paul & Page, In press). The present study has evaluated the dependence of PAF-acether induced cutaneous PPE responses on (a) circulating platelets, (b) local platelet accumulation and (c) the platelet release reaction. Radioisotopic methods have been used to quantitate PPE and PA responses in guinea-pigs (Paul & Page, In press).

a). Rabbit anti-guinea-pig platelet serum (APS) was prepared and used as described previously (Butler & Smith, 1981). Pretreatment with APS reduced the number of circulating platelets by >90% compared to animals treated with normal rabbit serum (NRS). Although PPE responses to PAF-acether (0.1-10 ng/site) in APS treated animals were reduced compared to the NRS controls, these reductions were not statistically significant (Student's t-test).
b). In four untreated guinea-pigs, PPE responses were significantly increased ($p < 0.001$) and PA significantly reduced ($p < 0.005$), in skin sites injected with PAF-acether (100 ng/site) + prostaglandin E1 (1 ug/site) when compared, using a paired t-test, to sites injected with PAF-acether alone.
c). Animals were pretreated intravenously with a drug combination known to inhibit the platelet release reaction (mepyramine, 0.2 mg/kg; methysergide, 0.2 mg/kg; indomethacin, 5 mg/kg; Vargaftig et al, 1982). Such animals did not show significantly reduced PPE responses to PAF-acether (0.1-100 ng/site) when compared with untreated animals (Student's t-test).

These results suggest that PAF-acether can induce PPE in guinea-pig skin independently of circulating platelets, platelet accumulation or platelet release products.

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U46619 STIMULATES PHASIC ACTIVITY IN RAT PORTAL VEIN. A POSSIBLE ROLE FOR TxA_2-RECEPTORS LINKED WITH A Ca^{2+}-GATING MECHANISM

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Rat portal vein has myogenic phasic activity which can be increased by some agonists without causing a sustained increase in tone (Hicks 1982); these effects are dependent on Ca2+-influx. This paper reports on the stimulant effects of the stable thromboxane A_2 (TxA_2)-receptor agonist U46619 (Bundy 1975) on phasic activity in the portal vein and the effects of the TxA_2-receptor antagonist EP045 (Jones et al 1982) on phasic responses induced by U46619 and other stimulants.

Portal veins from male Wistar rats (250-350g) were set up under 0.5g tension in physiological salt solution (PSS) containing propranolol (100 nM) and ascorbate (100 µM). Phasic response was calculated as the sum of individual phasic waves in response to agonists and expressed as a ratio of control phasic activity (Hicks 1982).

U46619 (0.5 nM-5 µM) was a potent stimulant of phasic activity and at high concentrations (10 µM) caused a contracture of the portal vein (0.43±0.08g), PGE_2 (0.1-10 µM) or $PGF_{2\alpha}$ (1-10 µM) were weak stimulants of phasic activity. EP045 (500 µM, 30 min) significantly ($P<0.01$) reduced spontaneous phasic activity (32.6±6.8%, n=6), antagonised phasic responses to cumulative additions of U46619, K^+, the α_2-adrenoceptor agonist UK14304 (Cambridge 1981) or NA, but did not antagonise phasic responses induced by phenylephrine (PE) or adrenaline (Fig 1). Phasic responses to U46619 were not significantly antagonised by combined treatment with prazosin (50 nM) and yohimbine (100 nM) but were antagonised by the Ca^{2+}-entry blocking drug, diltiazem (-logM, IC50 = 7.82±0.09).

The results indicate that U46619 is a potent stimulant of phasic activity in rat portal vein. These effects are probably as a result of TxA_2-receptor stimulation. The antagonist effects of EP045 against phasic responses to other stimulants might indicate that TxA_2-mechanisms are involved in a common Ca^{2+}-gating mechanism in this preparation. Alternatively, EP045 might have 'Ca^{2+}-antagonist' properties.

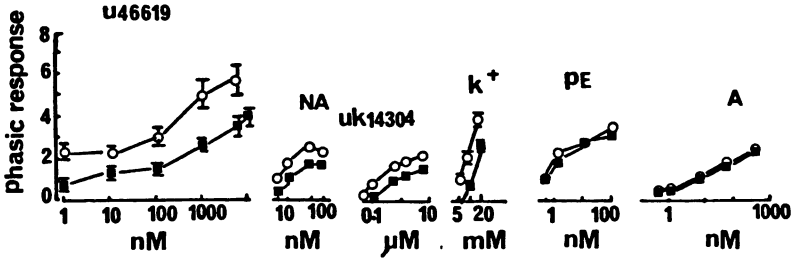

Figure 1: phasic responses induced by U46619 (n=8), K^+ (n=6); UK14304 (n=6), NA (n=6), PE (n=6) or A (n=6) before (o—o), or after treatment with EP045 (500 nM; 30 min ■—■).

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ACTIONS OF PROSTANOIDS ON THE RAT ANOCOCCYGEUS MUSCLE: ENHANCEMENT BY VERAPAMIL

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The rat anococcygeous muscle contracts to PGE_2 (threshold concentration = 5×10^{-8} M) and 11,9-epoxymethano PGH_2 (5×10^{-8} M), a thromboxane A_2 mimetic. EP 045, a thromboxane receptor antagonist (Jones et al, 1982) blocks the effect of 11,9-epoxymethano PGH_2 (pA_2 value = 6.67) but not that of PGE_2 or noradrenaline.

The sensitivity of the preparation to 11,9-epoxymethano PGH_2 is little affected by changes in external calcium concentration over the range 0.6 - 5.0 mM, but complete removal of external Ca^{++} reduces the maximum response by about 90%. Surprisingly, verapamil (1×10^{-6} - 5×10^{-5} M) enhanced the contractile effect of both 11,9-epoxymethano PGH_2 and PGE_2. In contrast contractions elicited by noradrenaline or K^+ were inhibited by verapamil. 6-Hydroxydopamine was used in vitro to produced sympathetic denervation. This procedure was without effect on the potentiating action of verapamil: pP_2 values for control and treated preparations were 5.03 and 5.23, respectively. The potentiating activities of other compounds are shown in Table 1. Nifedipine and flunarizine showed minimal potentiation at 10^{-5} M and inhibited 11,9-epoxymethano PGH_2 contractions at 10^{-4} M.

Table 1. pP_2 values for potentiation of 11,9-epoxymethano PGH_2 (mean ± s.e. mean, n = 4).

Compound	pP_2	Compound	pP_2
trifluoperazine	5.88 ± 0.07	propranolol	4.38 ± 0.11
amitriptyline	5.58 ± 0.21	penfluridol	4.30 ± 0.12
promethazine	5.39 ± 0.23	trifluoperazine sulphoxide	4.29 ± 0.15
verapamil	5.03 ± 0.19	trans-flupenthixol	4.01 ± 0.04
chlorpromazine	4.94 ± 0.02	D-600	3.83 ± 0.12
cis-flupenthixol	4.48 ± 0.10		

We can at this time only speculate about the mechanisms of the observed effects. It is of interest that the most active agent in our studies, trifluoperazine, is a potent inhibitor of calmodulin activation of the Ca^{++} ATPase pump (see Weiss et al, 1980). Further studies are in progress in relation to this area.

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THE SPONTANEOUSLY CONTRACTING PREGNANT RAT UTERUS AS A SCREENING TEST FOR PHOSPHOLIPASE A_2 INHIBITORS

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Prostaglandin synthesis and release increases markedly in the near term pregnant rat uterus leading to increased spontaneous contractile activity (Vane and Williams, 1973) which is inhibited by non-steroidal anti-inflammatory drugs (NSAID's) (Lewis et al, 1975). In the present study, we have utilised this preparation to evaluate agents acting at different target sites of phospholipid metabolism. In particular we have investigated the model as a screening test for phospholipase A_2 (PLA_2) inhibitors.

Uteri were removed from 18-22 day pregnant rats (AP1, 250-400g) and strips (3 x 1 cm) were suspended in Krebs solution (37°C, gassed 5% CO_2:95% O_2, 7 ml bath volume). Spontaneous contractions were recorded isotonically (load, 2g) and the bath fluid was changed every 20 minutes. After 2 to 3 cycles for equilibration drugs were added immediately following washout, with each concentration being in contact with tissue for 2 cycles. Tissue responses were recorded as the product of the number and mean height of contractions during the period of 10 to 15 minutes of the second cycle. Drug effects were expressed as IC_{50} values calculated from log dose-response curves in at least two experiments. Tissue responsiveness following drug blockade was checked by addition of PGE_2 (50 $ng.ml^{-1}$) which restored spontaneity in viable preparations.

Table 1 IC_{50} values (μM) for inhibition of spontaneous contractile activity in the near term pregnant rat uterus

NSAID's	IC_{50}	Steroids and PLA_2 inhibitors	IC_{50}
Indomethacin (4)	0.14	Dexamethasone (3)	1.2
Phenylbutazone (3)	7.1	Prednisolone (3)	7.3
Benoxaprofen (3)	34.5	Mepacrine (2)	3.7
BW 755C, base (5)	0.13	U-2021 (3)	23.8

Number of experiments shown in parentheses.

Results with the NSAID's were as expected from literature reports (Lewis et al, 1975; Higgs et al, 1979) but the steroids were more potent than previously reported (Lewis et al, 1975). This difference we attribute to increased overall drug contact time (2 cycles) which in the case of the steroids is necessary for the induction of adequate macrocortin synthesis (Blackwell et al, 1980). The inhibitory activity of the PLA_2 inhibitors mepacrine and U-2021 was apparent at concentrations lower than reported (240 and 160 μM respectively) for inhibition of hog pancreatic PLA_2 (Wallach and Brown, 1981).

We conclude that the near term pregnant rat uterus is a simple technique for detecting compounds that reduce prostaglandin output either by inhibition of cyclo-oxygenase or of PLA_2. Results must be interpreted with caution however since the preparation is particularly susceptible to compounds with non-specific spasmolytic activity.

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ST-SEGMENT CHANGES AND LACTATE AND K^+ PRODUCTION IN ISCHAEMIC MYOCARDIUM: EFFECT OF DIPYRIDAMOLE AND VERAPAMIL

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Ligation of a coronary artery elevates the S-T segment of the electrocardiogram (Maroko et al., 1971) and causes metabolic changes that are reflected in venous blood draining an ischaemic zone (Owen et al., 1970). The purpose of this study was to evaluate a model of temporary ischaemia in the anaesthetised dog that permits each animal to serve as its own control.

Twenty-seven mongrel dogs were anaesthetised and assigned to four treatment groups: control (vehicle), verapamil (0.25 mg/kg), verapamil (0.5 mg/kg) and dipyridamole (1 mg/kg). ST-segment changes were assessed using the technique of Maroko et al. (1971), employing 10 to 12 epicardial sites; the mean S-T segment elevation was determined before and at three times during occlusion. Lactate and potassium coronary venous levels were measured at 5-min intervals for 25 min before and after occlusion. Three 10-min coronary occlusions (LAD) were performed in the control group, and two occlusions were made in treated animals. Drug or vehicle were administered i.v. 5 min before the second occlusion. Successive occlusions were separated by 1-hr rest intervals.

The mean S-T segment elevation in control animals was marked and highly reproducible between the first and second occlusions. The S-T segment elevation during the third occlusion was somewhat decreased. At the low dose (0.25 mg/kg), verapamil significantly inhibited S-T elevation by about 50%. At the high dose (0.5 mg/kg), it induced haemodynamic changes such as hypotension, bradycardia, negative inotropism and A-V block. Ventricular extrasystoles were marked after coronary occlusion in these animals. Dipyridamole (1 mg/kg) provided no significant protection against S-T segment changes; at this dose level, dipyridamole is a potent coronary vasodilator but reduces blood flow in ischaemic zones (Parratt et al., 1980) and may even cause coronary steal (Becker, 1978).

Immediately after coronary occlusion, a change from lactate extraction to lactate production was observed, with a return to normal levels within 5 to 10 min of the end of occlusion. Levels during the second occlusion did not differ significantly from those during the first. There was also a marked increase in potassium in the blood draining the ischaemic zone. This was reproducible and did not vary significantly during the three occlusions. Verapamil (0.25 mg/kg) markedly reduced lactate (-79%) and K^+ (-60%) production; at the higher dose (0.5 mg/kg), which induced arrhythmias, a constant production of lactate and K^+ was seen in both venous and arterial blood. Dipyridamole did not modify K^+ efflux or lactate production.

Thus at an effective dose (0.25 mg/kg), verapamil inhibited S-T segment elevation and the production of both lactate and K^+. The higher dose of verapamil and the coronary dilator dipyridamole had no significant activity and, in some cases, appeared to intensify the ischaemia. The temporary occlusion model is both reliable and useful in the investigation of anti-ischaemic drugs. It allows animals to serve as their own controls and permits concomitant evaluation of S-T segment changes as well as lactate and K^+ production in the ischaemic zone.

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ASSESSMENT OF ADENOSINE AS THE CORONARY VASODILATOR MEDIATOR RELEASED FROM GUINEA-PIG HEARTS BY USE OF ADENOSINE DEAMINASE

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Adenosine is widely believed to serve as the mediator of coronary autoregulation and vasodilatation in response to hypoxia and cardiac hyperactivity (Berne, 1980). However, previous studies from this laboratory have shown that when donor and recipient hearts are perfused in series, the vasodilator material released from donor hearts stimulated by isoprenaline or histamine is not potentiated by the adenosine uptake inhibitor hexobendine, and was not therefore adenosine (Rothaul & Broadley, 1981). Using a similar technique, others have obtained evidence favouring adenosine since adenosine deaminase reversed the vasodilator response to released mediator (Schrader & Bardenheuer, 1981). We have therefore re-examined the effects of adenosine deaminase upon mediator released by histamine.

Guinea-pig donor and recipient hearts were perfused in series with Krebs-bicarbonate solution gassed with 5% CO_2 in O_2 at 38°C as described previously (Rothaul & Broadley, 1982). The pO_2 on entry to the donor was 500mm Hg and in the effluent fell to 120mm Hg which was regassed to 540mm Hg before entry to the recipient. In preliminary experiments, an infusion of adenosine deaminase (0.14ml min^{-1}, 15 U ml^{-1}) into the regassing chamber was found to abolish the vasodilator responses of recipient hearts to exogenous adenosine, the shift of the dose-response curve being 62.2-fold.

Donor and recipient hearts were exposed to histamine (1µg) which produced positive inotropic and chronotropic responses and a predominant coronary vasodilatation. The direct effects of histamine carried over in the effluent were antagonized in the recipient by a continuous infusion (0.14ml min^{-1}) of mepyramine (H_1, 40µg ml^{-1}) and cimetidine (H_2, 300µg ml^{-1}) into the regassing reservoir. The unchanged responses to histamine of the donor hearts were now followed by a coronary vasodilatation in the recipient, attributed to the release of vasodilator mediator. Adenosine (25µg) and acetylcholine (100ng) were added to the donor, producing coronary vasodilatation in the recipient without inotropic or chronotropic effects. Infusion of adenosine deaminase to the regassing chamber was then commenced and blockade of adenosine in the recipient confirmed. Administrations to the donor were then made in the following order: histamine, adenosine, histamine, adenosine and acetylcholine. The coronary vasodilator responses of recipient hearts, measured as the fall in perfusion pressure, were averaged and compared with the responses obtained before infusion of the deaminase. The mean (n=8 experiments) response to adenosine was virtually abolished from 9.2±2.0 to 0.16±0.16mm Hg, a reduction of 97.2±2.8%. The response to histamine-induced mediator release was also reduced, but by only 58.0±14.4%, from 4.5±0.5 to 1.9±0.6mm Hg.

Acetylcholine was included as a measure of any non-selective depression of vasodilator responses by deaminase and as a control for the viability of the preparation. The response was reduced by 22.1±12.5% from 8.9±1.2 to 6.6±0.9mm Hg. This was not significantly different ($P>0.05$) from the reduction of the response to vasodilator mediator released by histamine, some of which could therefore be attributed to these factors and not to deaminase activity. These results suggest that adenosine may serve as only one mediator of the coronary vasodilator response to histamine and that a further mediator has yet to be identified.

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DETERMINATION OF AGONIST AFFINITY IN SUPERSENSITIVE LEFT ATRIA OF GUINEA-PIGS PRETREATED WITH 6-OH DOPAMINE

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Chronic pretreatment of animals with 6-hydroxydopamine (6-OHDA) results in chemical sympathectomy and an increase in sensitivity of the effector organ to exogenous sympathomimetic amines (Kostrzewa & Jacobowitz, 1974). Reports of 6-OHDA-induced supersensitivity in cardiac tissue are variable (Haeusler, Haefely & Thoenon, 1969; Brus, Hess & Jacobowitz, 1970) and where it is observed, may be of pre-junctional origin due to inhibition of neuronal uptake (Nadeau, de Champlain & Tremblay, 1971). The present study was undertaken firstly to establish post-junctional cardiac supersensitivity to sympathomimetic amines and secondly to determine whether it was due to an increase in agonist affinity.

Male guinea-pigs were pretreated with 6-OHDA ($460 mgkg^{-1}$) given by intracardiac injection in 6 divided doses over 20 days. Control animals were sham injected with vehicle for the same period. Animals were then killed and the left atria removed from the heart and secured to bipolar electrodes for pacing at 2Hz with pulses of 5ms duration and threshold voltage (+ 50%). Atria were suspended in Krebs-bicarbonate solution at 38°C gassed with 5% CO_2 in O_2 and containing metanephrine (10μM) and phentolamine (5μM). Cumulative dose-response curves for the tension increase in response to isoprenaline were obtained, followed, after washout, by a curve to salbutamol. Atria from 6-OHDA-pretreated animals were supersensitive as shown by the significantly lower ($P<0.05$) EC_{50} values for isoprenaline (1.33 (0.22-8.18)nM, n=5) than after sham injection (12.5 (2.96-53.0)nM, n=4). Furthermore, salbutamol was a partial agonist, the maximum tension increase being 23.0 ± 2.1% of the isoprenaline maximum in atria from sham injected animals and after 6-OHDA this was significantly raised ($P<0.05$) to 38.2 ± 4.2%.

No supersensitivity to histamine occurred, the EC_{50} value after 6-OHDA (1.19 (0.3-4.7)μM, n=5) and sham injections (1.18 (0.7-2.1)μM, n=5) not differing significantly ($P>0.05$). The supersensitivity to isoprenaline and salbutamol could therefore be considered post-junctional, since neither are taken up into sympathetic neurones and specific for the β-adrenoceptor.

Next, the dissociation constants (K_A values) of a third agonist, orciprenaline, were calculated by constructing dose-response curves before and after washout (2h) of the irreversible β-adrenoceptor antagonist Ro 03-7894 ($3.8x10^{-4}$M), following a 30 min incubation. K_A values were determined as described previously (Broadley & Nicholson, 1981). Atria from 6-OHDA pretreated animals were supersensitive to orciprenaline, the EC_{50} values (0.66 (0.39-1.1)μM, n=8) being significantly less ($P<0.05$) than after sham injections (2.22 (1.3-3.8)μM, n=9). However, the mean K_A value obtained in supersensitive atria (5.63±1.13μM, n=4) was not significantly different ($P>0.05$) from the K_A value after sham injections (6.80±3.96μM, n=4). These results suggest that the post-junctional supersensitivity to β-adrenoceptor agonists occurring after chronic pretreatment with 6-OHDA is not due to an elevation of the affinity of agonists for the cardiac β-adrenoceptor.

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SPECIFICITY OF ACTION OF RANITIDINE AS A HISTAMINE H_2-RECEPTOR ANTAGONIST

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Ranitidine is a selective histamine H_2-receptor antagonist with a calculated K_B value at H_2-receptors in the guinea-pig right atrium of 0.63 (0.34 - 0.98) x 10^{-7}M (Daly & Stables, 1980). It has no effect on bethanechol or histamine H_1-receptor mediated responses of the guinea-pig ileum at 3 x 10^{-4}M, and, unlike cimetidine, shows little or no affinity for cytochrome P450 or androgen receptors (Brittain et al, 1982). However, Bertaccini & Coruzzi (1982) recently reported that ranitidine could cause cholinergic effects such as contraction of isolated intestinal smooth muscle, and potentiation of bethanechol-induced salivary secretion in the rat. The present study is a further evaluation of the action of ranitidine on some preparations containing muscarinic receptors.

The spasmogenic activity of ranitidine was investigated on isolated preparations of guinea-pig ileum or rat lower oesophageal sphincter (L.O.S.) suspended in a modified Krebs solution at 32°C and gassed with 95% O_2/5% CO_2. Concentration-response curves to acetylcholine were repeated until constant, and 30 min later a concentration-response curve to ranitidine was obtained. Ranitidine caused contractions of both preparations at the relatively high concentrations of 1 x 10^{-5} to 1 x 10^{-3}M on guinea-pig ileum (n = 4), and 3 x 10^{-6} to 1 x 10^{-3}M on rat L.O.S. (n = 8). These responses could be inhibited by atropine (3 x 10^{-8}M), suggesting an involvement of muscarinic receptors. This may not be a direct muscarinic effect since ranitidine only produced contractions of the rat L.O.S. in preparations that had been previously exposed to acetylcholine, and was virtually ineffective if given before acetylcholine, or in preparations in which bethanechol was tested instead of acetylcholine.

Ranitidine, at concentrations up to 3 x 10^{-4}M, had no cholinomimetic effect on the guinea-pig right atrium. In beagle dogs, anaesthetised with chloralose and urethane, ranitidine had no effects on blood pressure or heart rate at 1 mg kg^{-1} i.v., a dose 40 times the antisecretory ED_{50} of 0.027 (0.010 - 0.049) mg kg^{-1} against histamine induced gastric acid secretion. Furthermore in the dog, in contrast to data reported for the rat, ranitidine (up to a dose of 10 mg kg^{-1} i.v.) had no effects on salivary secretion elicited by infusion of a submaximal dose of bethanechol (3 µg kg^{-1} min^{-1} i.v.). Since the weak cholinomimetic action of ranitidine is limited to particular tissues and/or animal species it is most unlikely to have clinical relevance.

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2-CHLOROADENOSINE LOWERS BLOOD PRESSURE IN THE CONSCIOUS DOG WITHOUT REFLEX TACHYCARDIA

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Vasodilators typically evoke reflex increases in heart rate and in plasma renin activity (Koch-Weser, 1974). Purines such as adenosine are vasodilators but they also inhibit the release of noradrenaline from the adrenergic nerves (Verhaeghe, Vanhoutte and Shepherd, 1977). Consequently they would be expected to evoke smaller increases in heart rate and plasma renin activity than do conventional vasodilators. This hypothesis has been tested by comparing the effects of a stable adenosine analogue (2, chloroadenosine) with that of hydralazine in the conscious normotensive dog.

Two groups of mature male beagle dogs(12-18kg)with indwelling carotid catheters were used. Blood pressure was recorded directly via the carotid catheter with heart rate derived electronically from the blood pressure signal. Samples of arterial blood were taken for determination of plasma renin activity (PRA) by radioimmuno-assay. Control haemodynamic readings and two blood samples were taken during a thirty minute period prior to dosing and further readings and blood samples taken up to five hours thereafter. Control parameters were not significantly different between the two groups of dogs (Table 1).

Both hydralazine (20mg/kg p.o. n=4) and 2-chloroadenosine (2mg/kg p.o. n=4) produced significant decreases in diastolic blood pressure (DBP) from 1-3 hours after dosing. Small decreases in systolic blood pressure also occurred(Table 1). The decreases in DBP evoked by hydralazine and 2-chloroadenosine were not significantly different. Hydralazine produced a significant tachycardia 1-5 hours after dosing (Table 1). There was no significant change in heart rate (HR) in the dogs treated with 2-chloroadenosine. Both compounds increased PRA (Table 1). This was significant 1-3 hours after dosing hydralazine. In dogs treated with 2-chloroadenosine a significant increase in PRA was only observed 1 hour after administration. The increment in PRA evoked by hydralazine was significantly greater than that produced by 2-chloroadenosine at 1 and 3 hours after dosing ($P<0.05$).

Table 1	Systolic BP(mmHg)	Diastolic BP(mmHg)	Heart Rate (bts/min)	Plasma Renin Activity (ng/ml/hr)
2-chloroadenosine(2mg/kg p.o. n=4)				
Control	130 ± 4.2	70 ± 4.1	90 ± 4.1	1.0 ± 0.20
1 hour	120 ± 3.5*	55 ± 7.4*	93 ± 14.5	2.7 ± 0.74*
3 hours	110 ± 7.4	54 ± 3.8*	93 ± 2.5	1.2 ± 0.21
5 hours	116 ± 6.9	58 ± 3.2	81 ± 5.2	1.3 ± 0.19
Hydralazine(20mg/kg p.o. n=4)				
Control	134 ± 4.3	71 ± 5.5	78 ± 4.3	0.8 ± 0.17
1 hour	120 ± 2.0*	59 ± 3.8*	155 ± 21.8*	10.9 ± 3.43*
3 hours	123 ± 4.8	61 ± 4.4*	173 ± 23.6*	15.3 ± 5.13*
5 hours	121 ± 2.4*	64 ± 6.3	161 ± 22.5*	12.3 ± 7.23

Data is expressed as mean ± SEM. *Significantly different from control $P<0.05$

These results demonstrate that equieffective hypotensive doses of hydralazine and 2-chloroadenosine have different effects on HR and PRA. The lack of tachycardia and the minimal increase in PRA observed in dogs treated with 2-chloroadenosine indicates that the compound attenuates the increase in sympathetic tone evoked by hypotension. This could be due to presynaptic inhibition of noradrenaline release from the sympathetic nerves. However, direct effects of the purine on the heart and the kidney cannot be discounted.

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ADRENERGIC NEURONE BLOCKING PROPERTIES OF RS-51324

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RS-51324 (DL-4(5)-(2,6 dichlorophenyl)-2-methoxycarbonyl-amino-4,5 dihydro-imidazole) has previously been reported as a membrane-pump inhibiting compound with possible antidepressant activity (Wallach et al, 1981). However, when administered to healthy human volunteers, the compound produced a delayed but sustained fall in diastolic blood pressure (BP) in both the supine and standing positions (Harris, personal communication). Since this compound was shown to possess only mild transient effects on cardiovascular function following intravenous administration to anaesthetised cats (Wallach et al, 1981), it was of interest to determine the mechanism of the hypotensive response to RS-51324 which had been seen in man.

Male Spontaneously Hypertensive Rats of the Okamato Strain (SHR) with systolic BP of >170 mmHg (1 mmHg ≃ 133 Pa) were used. Systolic BP and heart rate (HR) were recorded from conscious SHR by a modification of the tail cuff method (Friedman & Freed, 1949). RS-51324 over the dose range 10-100 $mg.kg^{-1}$ p.o. caused dose-related falls in systolic BP in SHR which attained a maximum effect between 6-12 h post-dose. The antihypertensive effect was prolonged, lasting longer than 24 h, with recovery to pre-dose levels at 48 h. The antihypertensive effect was associated with little or no change in HR.

To determine whether the fall in BP was associated with an inhibition of the peripheral sympathetic nervous system (SNS), Sprague Dawley rats were pithed and the complete sympathetic outflow of the spinal cord was stimulated by the method of Gillespie et al (1969). RS-51324 (100 $mg.kg^{-1}$ p.o.) or vehicle (N/100 HCl 10 $ml.kg^{-1}$ p.o.) were administered 8 h before spinal stimulation. BP responses to spinal stimulation were severely inhibited in rats receiving RS-51324 compared with control animals. The pressor response to exogenous noradrenaline was markedly potentiated when compared with control animals.

Dexamphetamine (10 $mg.kg^{-1}$ i.v.), administered after the block of the SNS was established, produced a reversal of the inhibition of the BP response to spinal stimulation, with return to control values.

RS-51324 did not inhibit spinal stimulation following administration of 10 $mg.kg^{-1}$ i.v. to pithed rats indicating that the compound itself was not an adrenergic neurone blocker. However, the primary amine of RS-51324 (RS-58550) was noted as a metabolite in man and this compound, previously described by Matier et al (1973), has antihypertensive and adrenergic neurone blocking properties itself. This compound on administration (10 $mg.kg^{-1}$ i.v.) to pithed rats produced enhancement of the pressor response to exogenous noradrenaline and an inhibition of the pressor response to spinal stimulation which was reversed by administration of dexamphetamine (10 $mg.kg^{-1}$ i.v.).

It is concluded that RS-51324 is a potent, long-acting antihypertensive agent in SHR and that at the time of peak activity, a metabolite of the compound displays potent adrenergic neurone blocking properties.

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SHORT-TERM INFUSION OF ANGIOTENSIN II DOES NOT STIMULATE SYSTEMIC PROSTAGLANDIN I_2 SYNTHESIS IN CONSCIOUS DOGS

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Angiotensin 11 (All) is a potent vasoconstrictor. It has been suggested that it may stimulate the release of vasodilator prostaglandin I_2 (PGI_2) from the vasculature. For example infusion of high doses of All into the isolated rat mesenteric vasculature (Dusting et al, 1980) and renal circulation of anaesthetised dogs (Shebuski & Aiken, 1980) was reported to release PGI_2. The present study aimed to clarify the physiological significance of these findings. Urinary excretion of 2,3 dinor 6 keto $PGF_{1\alpha}$ (a major metabolite of PGI_2) provides a useful measure of systemic levels of PGI_2 and in this study has been used to measure systemic PGI_2 synthesis during and after infusion of All.

6 female dogs were prepared with an electromagnetic flow probe around the left renal artery and catheters in the aorta and inferior vena cava. On the day of the studies the bladder was catheterised for collection of urine and a modest water diuresis was induced by infusion of dextrose (5g/100ml) DTPA was infused continuously to permit measurement of glomerular filtration rate (GFR). Systemic BP and renal blood flow (RBF) was monitored throughout. Urine was collected at 30 min intervals. The first 90 min constituted a control period, followed by a 3 hour infusion of All (15ng/kg/min) and then a 1 hour recovery period. Each animal, in a separate study, also received a continuous infusion of saline (140mM/1) as a further control. Plasma and urinary concentrations of 2,3 dinor 6 keto $PGF_{1\alpha}$ were measured using a stable isotope dilution method utilising negative ion chemical ionisation mass spectrometry.

During All infusion BP increased from 99.2 ± 4.6 to 129.7 ± 5.7mmHg ($P < 0.05$) and remained elevated throughout the infusion. RBF was decreased from 213 ± 6.3 to 138 ± 28ml/min by All infusion but like the BP returned to control values within 1 hour of stopping the infusion. GFR was decreased within 30 min of starting the All infusion (80.4 ± 6.4 to 65.3 ± 8.0ml/min, $P < 0.05$) but continued to further decrease during All infusion. Plasma All increased from 59.5 ± 9.4 to 188 ± 61pg/ml ($P < 0.05$) and remained elevated during the infusion. Urinary dinor 6 keto $PGF_{1\alpha}$ excretion was 1.2 ± 0.2ng/30 min during the control period and the same after 150 min infusion of All. Excretion during the recovery period (1.7 ± 0.4ng/30 min) was similar to that during the control period. Plasma dinor 6 keto $PGF_{1\alpha}$ was also unchanged before (11.3 ± 2.5pg/ml, n=5) and during (9.1 ± 1.0pg/ml, n=4) the All infusion.

Despite the significantly elevated plasma concentration of All and the associated haemodynamic changes, there was no significant change in urinary excretion or plasma level of dinor 6 keto $PGF_{1\alpha}$ suggesting that All does not stimulate systemic PGI_2 synthesis in normal conscious dogs.

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A COMPARISON OF THE HYPOTENSIVE ACTIVITY AND IN VITRO AND IN VIVO α_1-ADRENOCEPTOR ANTAGONIST PROPERTIES OF PRAZOSIN, TRIMAZOSIN AND DOXAZOSIN IN THE RABBIT

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The quinazoline derivatives Prazosin (P), Doxazosin (D) and Trimazosin (T) have been reported to possess selective peripheral α_1 adrenoceptor antagonist properties in animals (Kamarat Ali et al 1980; Timmermans et al 1980) and in man (Singleton et al 1980). This study compares depressor actions, antagonist properties in vivo and in vitro, and radioligand binding properties in male New Zealand white rabbits.

In vivo experiments were performed in conscious rabbits with direct recording of blood pressure (MAP). Groups of 7-10 were studied in each experiment and results expressed as mean ± SD. Statistical analysis was by repeated measures ANOVA for MAP and heart rate and by Student's 't' test for paired data for dose ratios from pressor responses.

In vitro ligand binding to membranes from rabbit cerebral cortex with ^{3}H prazosin (α_1) and ^{3}H clonidine (α_2) showed P and D were equipotent at displacing the radioligand ^{3}H prazosin (IC_{50} 8.9×10^{-8} and 5.2×10^{-8}M respectively)while T was less potent (IC_{50} 5.4×10^{-6}M). In all cases IC_{50} to displace ^{3}H clonidine was $>10^{-5}$M.

In vitro spiral strips of thoracic aorta, showed a parallel dose related shift to the right of the contractile response to phenylephrine after all 3 agents. The order of potency was P > D > T.

In vivo P, D and T intravenously caused dose-dependent parallel shifts to the right of the phenylephrine (0.001-0.3 mg/kg) pressor dose-response curves. In preliminary dose-ranging experiments, 0.1 mg/kg P and 0.3 mg/kg D caused similar degrees of α_1 antagonism. T 3 mg/kg caused a profound late (4-6 hrs) fall in MAP with only modest $\alpha_1$antagonism. The doses used in subsequent studies were P 0.1 mg/kg, D 0.3 mg/kg and T 3 mg/kg. The maximum shift of the pressor response curve occurred after 15 mins with P (dose ratio 3.8 ± 1.4) followed by a gradual recovery. With D, α_1 blockade after 15 mins was similar (3.0 ± 0.6) to P but was greatest at 4 hrs (4.3 ± 2.7). T produced a smaller shift of the pressor dose response curve (2.2 ± 0.4 at 15 mins) and 1.8 ± 0.5 at 4 hrs when the greatest fall in MAP was observed. Thus, in vivo α_1 blocking activity was P > D > T with D causing a paradoxical late α_1 blockade.

The magnitude of the early (15 min) blood pressure fall at the doses used was P > D > T while at later (4 hrs) times, the order was reversed (T > D > P).

The early hypotensive effect appears to correlate well with in vivo and in vitro α_1 blockade. However, whereas the later fall in MAP with D correlates with increased α_1 blockade the later fall in MAP with T may not be related to α_1 blockade. It is likely that both D and T form active metabolites which in the case of T but not D may lower blood pressure by an α_1 receptor independent mechanism.

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EFFECTS OF PIPROFUROL ON SINUS NODE FUNCTION OF DOGS' HEARTS

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Piprofurol is a new Ca^{2+} antagonist drug which has been shown to depress sinus node automaticity in isolated sinus node preparations of rabbit heart (Pourrias and Santamaria, 1983). To study the effects of piprofurol on sinus node function (SNF), experiments were done on "in situ" dogs'hearts.

Mongrel dogs of either sex (15-20 kg) were anaesthetized with Pentobarbital (30 mg/kg I.V.) intubated and artificially ventilated (room air + 50 % O_2). Sinus cardiac rate (P-P interval), corrected sinus node recovery time (CSNRT) (Mandel et al., 1972), and sinus atrial conduction time (SACT) (Narula et al., 1978) were assessed from standard ECG lead II and intracavitary electrical recordings. In order to directly measure SACT, epicardial sinus nodal and right atrial electrograms were recorded in another set of experiments.

Piprofurol, at the doses of 0.125 ; 0.175 and 0.25 mg/kg I.V., did not change sinus node function on dogs'hearts with intact autonomic nervous system. After autonomic blockade (atropine, 0.04 mg/kg, and propranolol 0.2 mg/kg) (Jose and Collison, 1970) I.V. piprofurol, at the same doses, significantly decreased P-P interval ($p < 0.01$) and increased CSNRT ($p < 0.005$). Piprofurol administered into the sinus node artery at the doses of 1 ; 5 ; 10 ; 25 and 50 mg/kg depressed, in a dose-dependent manner, sinus node automaticity ; once sinus automaticity was recovered sinus node function was still depressed for several minutes.

Our findings suggest that piprofurol intrinsically depresses sinus node function ; nevertheless, on "in situ" dogs'hearts experiments the autonomic modulation of the sinus node counteracts the action of the drug.

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THE EFFECTS OF PIPROFUROL ON SPONTANEOUS ELECTRICAL ACTIVITY OF ISOLATED RABBIT SINUS NODE

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Piprofurol has ben showed to have calcium antagonistic properties on vascular smooth muscle and ventricular myocardium (Pourrias et al., 1982). The effects of piprofurol have been further investigated in rabbit isolated sinus node in which the slow inward current carried, at least in part by calcium, is responsible of the upstroke of the action potentials and may play a role in pacemaking mechanism (see review of H.F. Brown, 1982). Preparations including a small part of the right auricle, the crista terminalis and the sinus node region have been perfused (10 ml/min) with Tyrode solution containing either 0.11 ; 0.22 ; 0.44 ; 0.88 μM of piprofurol. Action potentials were recorded with conventional microelectrodes.

Piprofurol decreased concentration-dependently the resting potential, total amplitude, slow diastolic depolarization of the action potential and the frequency of beating. The decrease of the amplitude is indicative of a decrase of the slow inward current. The duration of the action potential was not modified at any concentration of piprofurol (Table 1).

As the decrease of threshold potential was always equal or less than that of the resting potential, the slowing of the frequency could be attribute solely to the decrease of the diastolic depolarization. A part of this effect could be also the consequence of the inhibition of the slow inward current.

When verapamil was perfused at 0.11 ; 0.22 ; 0.44 and 0.88 μM, the same concentration-dependent effects appeared, but they were less pronounced and in addition verapamil increased the duration of the action potential, an effect which participated to the decrease of the frequency of beating.

In contrast diltiazem did not induced concentration-dependent decrease of the frequency of beating or of the slow diastolic depolarization in the range 0.11-0.44 μM and these variations were smaller than those of piprofurol.

These results are in agreement with the previously demonstrated calcium antagonistic properties of piprofurol and show that it differs in some way from other calcium inhibitors.

Table 1 : Effects of Piprofurol on sinus node (differences from control).

Piprofurol (μM)	RP mv (%)	Amp mv (%)	Diast.dep. mv/s (%)	Freq. bts/min (%)
0.11	- 5.7 (-8)	-17 (-22)	-19.9 (-23)	-13 (-9)
0.22	- 5 (-7)	-18 (-23)	-23 (-28)	-18 (-12)
0.44	- 9 (-14)	-26 (-33)	-24 (-37)	-25 (-19)
0.88	-19 (-27)	-42 (-54)	-33 (-58)	-35 (-28)

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ANTIDIPSOGENIC EFFECT OF β-PHENYLETHYLAMINE IN THE WATER-DEPRIVED RAT

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Previous work has revealed that β-phenylethylamine (PEA) at 50 or 100 mg/kg significantly reduces 24h food-intake in nondeprived rats (Dourish, 1982). The aim of the present study was to examine the possibility that PEA may also affect regulatory drinking responses. PEA is an endogenous constituent of the human and rodent brain (Durden et al, 1973; Philips et al, 1978), and may play an important part in the neural control of ingestional responses.

Forty-eight adult, male Wistar rats (250-350g) were housed individually and thoroughly adapted to a 23h daily water-deprivation schedule. The animals were allocated to 6 equal groups, which received 0, 3.25, 6.25, 12.5, 25.0 and 50.0 mg/kg PEA HCl respectively, immediately before the return of water to the home cage. Isotonic saline served as vehicle, and injections were by i.p. route. Water intake (ml) was measured at 15 min intervals during 1h period. A one-way ANOVA revealed a significant PEA effect during the first 15 min period ($p<0.001$), and trend analysis confirmed a linear effect of PEA dose on the level of water intake ($p<0.001$). Compared with the control group, PEA at 25.0 and 50.0 mg/kg produced significant reductions in water consumption ($p<0.05$ and $p<0.01$ respectively). During the latter 45 min of the test, there occurred a secondary hyperdipsia, particularly in animals treated with 50.0 mg/kg.

One week later the effects of PEA on spontaneous activity were investigated in the same groups of water-deprived animals, using an automated activity-recording system (Opto-Varimex Minor, Columbus Instruments), controlled by a microprocessor and microcomputer. Behavioural observation supplemented the automated recording. PEA exerted significant effects on total horizontal activity, ambulation and vertical activity (F values significant at $p < 0.01$ in each case). Dunnett's test indicated that compared with control animals, PEA at 6.25 mg/kg had no effect on total horizontal activity, ambulation or vertical activity; PEA at 12.5 mg/kg significantly enhanced total horizontal activity ($p < 0.01$); PEA at 50.0 mg/kg markedly reduced ambulation, total horizontal and vertical activities.

These data indicate that PEA can depress drinking in water-deprived rats. At 12.5 mg/kg, PEA stimulated horizontal activity and did not affect drinking. At the larger doses (25.0 and 50.0 mg/kg), PEA produced a general attenuation of drinking and activity. This coincided with the induction of a behavioural stereotypy syndrome, observed in the present study and previously (Dourish, 1981), characterised by a reduction in grooming and an increase in sniffing, lateral head-movements, splayed-hindlimbs, forepaw padding, and hyperreactivity.

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CENTRAL SITES OF ACTION OF PIRIBEDIL IN THE INDUCTION OF YAWNING, STRETCHING AND SEXUAL EXCITEMENT IN THE MALE RAT

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Systemic administration of muscarinic agonists, or of low doses of the dopamine agonists, piribedil (PB) and apomorphine, produce in male rats a behavioural syndrome characterised by sedation, excessive yawning, and sexual arousal (Dourish & Cooper, 1981; Gessa et al, 1966; Urba-Holmgren et al, 1977; Mogilnicka & Klimek, 1977). The present study attempted to identify a central site of action of PB linked to the production of the behavioural syndrome. Adult, male, Sprague-Dawley rats were implanted with bilateral guide cannulae aimed at either the caudate nucleus (A 0.11, L 0.2, V-0.55 cm) or the nucleus accumbens (A 0.34, L 0.17, V-0.62 cm); Konig & Klippel stereotaxic co-ordinates, using bregma as a zero-point. After surgery, the rats were habituated to perspex test chambers. They were given a bilateral injection of 100 µg PB methane sulphonate in 1µl of distilled water and, on a second occasion a bilateral injection of 1 µl isotonic saline, in a counterbalanced order.

During a 60 min test 2 observers rated a variety of qualitatively-distinct behavioural responses as previously described (Dourish, 1982). In some cases scopolamine (1 mg/kg, 25 min after PB) or haloperidol (0.025 mg/kg, 30 min prior to PB) were administered i.p. in an attempt to block the PB syndrome. Bilateral injection of PB into the caudate nucleus induced recurrent yawning, stretching, grooming, chewing and penile erection in 10 out of 12 rats tested (mean number of yawns, 28.4 ± 3.6). The onset of this syndrome was 5-10 min post-injection and the effects lasted for 40-50 min. After bilateral intracaudate injections of an equivalent volume of saline the mean number of yawns observed was 2.9 ± 1.79 during the same period. Rats tested with intraaccumbens PB exhibited a mean of 11.66 ± 2.56 yawns, a response which was significantly different from that induced by an equivalent volume of saline (mean 0.00). Administration of scopolamine or haloperidol abolished the syndrome induced by intracaudate PB application. The present data demonstrate that PB itself is centrally active and produces a syndrome of yawning, stretching and sexual excitement when applied to the caudate nucleus and nucleus accumbens of the rat. A previous investigation failed to identify any effects of intracaudate administration of PB (Costall & Naylor, 1974). Our results indicate that DA neurons may play an integral role in the mediation of sleep-related behaviour and sexual activity. Since sedative effects of DA agonists may relate to the inhibition of DA neurons, it is possible that yawning follows the release of cholinergic neurons from DA inhibition. This interpretation fits the present data, and a previous finding that the syndrome can be elicited by muscarinic agonists (Urba-Holmgren et al, 1977).

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THE ROLE OF THE SUBSTANTIA INNOMINATA-VENTRAL GLOBUS PALLIDUS IN MEDIATING N. ACCUMBENS-EVOKED HYPERACTIVITY

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Neuroanatomical studies on rats revealed a dense innervation of the rostroventral part of the globus pallidus (GP) beneath the anterior commissure by N.accumbens (NA) efferents (Williams et al, 1977). A later study (Walaas & Fonnum,1979) has shown that GABAergic projections originate in NA and terminate partly in the rostroventral GP, but mainly in the adjacent substantia innominata (SI). We have examined the roles of the SI and the rostroventral GP in mediating the hyperactivity produced by injection of the dopamine receptor agonist ADTN into the NA (Elkhawad & Woodruff, 1975).

ADTN (10μg) caused prolonged locomotor stimulation after a 1h latent interval when injected bilaterally via cannulae into the NA of female Sprague Dawley rats (180g). Peak hyperactivity, measured by placing rats singly in activity cages, occurred 4h after ADTN injection.

Bilateral electrolytic lesions (2 mA, 15 s) were made in SI (A 6.9, H -2.1, L 2.7; König & Klippel 1963) and the adjacent rostroventral GP (A 7.2, H -1.9, L 1.5). Both types of lesions, which were not entirely restricted to the target area, reduced ADTN-induced locomotor stimulation. During the period 3-5h after ADTN, the mean activity scores (counts /30 min) were: normal rats 1201 ± 179 (n=6); SI lesioned 487 ± 79 (n=6, $P < 0.05$); ventral GP lesioned 448 ± 86 (n=6, $P < 0.05$).

Picrotoxin (0.25 - 0.5 μg), when injected bilaterally into SI of 6 cannulated rats, provoked intense hyperactivity lasting up to 3h. A similar, but less intense stimulation of activity was produced by picrotoxin in ventral GP.

Cryostat-cut coronal sections of rat brain were used to visualise autoradiographically $GABA_A$ and $GABA_B$ receptors (Wilkin et al, 1981) in the SI and ventral GP. Both receptor subtypes were present in the 2 brain areas. Counts of silver grains revealed that the ratio of $GABA_A$: $GABA_B$ receptors was 1 : 0.6 in SI and 1 : 1.5 in ventral GP.

The effect of local injections of GABA drugs in SI on ADTN-induced hyperactivity was examined. Muscimol (10ng), injected bilaterally in SI 1h after ADTN in NA, had no significant effect on the peak hyperactivity. By contrast, the $GABA_A$ receptor agonist isoguvacine (0.125 - 2 μg) in SI caused a dose-related inhibition of the locomotor stimulation (e.g. 0.5 μg reduced the 3-5h activity score from 1201 ± 179 to 705 ± 87). The putative $GABA_B$ receptor agonist baclofen (0.25 μg) in SI entirely prevented ADTN-induced hyperactivity.

These results indicate that GABAergic mechanisms in the substantia innominata and subcommissural rostroventral GP are involved in mediating the hyperactivity response evoked from the NA.

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