HETEROGENEITY OF MUSCARINIC BINDING SITES IN RAT BRAIN, SUBMANDIBULAR GLAND AND ATRIUM.

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Until recently the subclassification of M_1- and M_2-muscarinic receptors was based upon the preferential M_1-affinity of pirenzepine (Hammer et al., 1980). Owing to the discovery of AF-DX 116 (11-2[[2-[(diethylamino)methyl]-1-piperidinyl]acetyl]-5,11-dihydro-6H-pyrido[2,3-b][1,4]benzodiazepin-6-one), it appeared that the M_2-receptor subclass can no longer be considered a homogenous population (Hammer et al., 1986). Moreover, Eglen and Whiting (1986) suggested to subclassify the M_2-receptors in the ileum and the heart as M_2a- and M_2b-receptors, respectively.

To further investigate the M-receptor heterogeneity, we compared the affinity of atropine, pirenzepine, dicyclomine, 4-diphenylacetoxy-N-methylpiperidine methbromide (4-DAMP) and AF-DX 116 for M-receptor binding sites in rat cerebral cortex, hippocampus, cerebellum and submandibular gland and the heart (atria). From displacement of specific ^{3}(H)-N-methylscopolamine (^{3}(H)-NMS; 0.4 nmol/l; 85 Ci/mmol) or ^{3}(H)-pirenzepine (^{3}(H)-PZ; 2 nmol/l; 76 Ci/mmol), pKi-values were determined. Specific binding was determinated with 1 µmol/l dexetimide.

Table 1. Comparison of pKi values for muscarinic antagonists in rat heart, submandibular gland and brain.

	pKi-values			
	^{3}(H)-PZ	^{3}(H)-NMS		
	hippocampus	heart	submandibular gland	cerebellum
atropine	9.20	8.87	8.78	8.67
pirenzepine	7.43	5.86	6.38	6.09
dicyclomine	8.00	6.46	7.59	6.74
4-DAMP	8.74	7.47	8.48	7.91
AF-DX 116	6.09	6.71	5.30	6.44

Values represent mean of four separate experiments in duplo. S.E.M. < 5%.

Atropine behaved as a non-selective high affinity ligand in all tissues studied. Pirenzepine had high affinity for the hippocampus and low affinity in the heart, submandibular gland and cerebellum, dicyclomine and 4-DAMP displayed high affinity for the hippocampus and submandibular gland and low affinity for the heart and cerebellum. AF-DX 116 had high affinity for the heart, intermediate affinity for the hippocampus and cerebellum and low affinity for the submandibular gland. In contrast to pirenzepine, the ^{3}(H)-NMS displacement curves of dicyclomine and 4-DAMP indicated an apparent homogenous population of high affinity binding sites in the cerebral cortex.

We conclude that the M-binding sites in the hippocampus, heart and submandibular gland are distinct subpopulations and may be labelled M_1, M_2 and M_3, respectively. In the cerebellum, the major subpopulation belongs to the M_2-type. The cerebral cortex contains a mixed population of M_1 and M_3-muscarinic binding sites.

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We would like to thank Dr. R.B. Barlow for the gift of 4-DAMP.

SHORT-TERM DESENSITISATION AND ITS APPLICATION TO THE ESTIMATION OF AGONIST AFFINITY CONSTANTS.

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Short-term desensitisation of the ileal muscarinic receptor has been shown to result in a dextral shift in the concentration-responses curve and a depression the maximum (Siegel et al., 1984). These effects are very similar to those observed following partial receptor inactivation after exposure to an irreversible antagonist (Furchgott and Bursztyn, 1967). It is possible therefore that desensitisation may be used in an analogous fashion to receptor inactivation to estimate agonist affinity constants. However, for such null methods of analysis to be employed, the procedure must affect the receptor alone and not the stimulus-response coupling (Leff et al., 1985). The aim of the present study was to compare the values obtained using desensitisation and receptor inactivation as estimates of agonist affinity constants.

All studies were undertaken using guinea-pig ilea. Non-cumulative concentration-response curves were constructed to muscarinic agonists before and after exposure to either carbachol (1×10^{-5}M; 60 minutes) to produce desensitisation or phenoxybenzamine (3×10^{-6}M; 20 minutes) to produce receptor inactivation. After each of these procedures the tissues were washed once. Equiactive concentrations from pre- and post desensitisation or inactivation curves were then plotted in a double reciprocal fashion, and the agonist affinity constants estimated using the method of Furchgott and Bursztyn (1967). The results are shown in Table 1.

AGONIST	DESENSITISATION	INACTIVATION
Carbachol	6.72 ± 0.09	4.52 ± 0.08
Acetylcholine	6.40 ± 0.05	5.30 ± 0.02
Methacholine	5.66 ± 0.07	4.81 ± 0.06
Arecoline	5.92 ± 0.03	4.82 ± 0.05
APE	7.70 ± 0.06	6.72 ± 0.04

Table 1. Agonist affinity constants (-log K_A) estimated using desensitisation or receptor inactivation techniques. Values are mean ± SEM, n = 4-6. APE = arecaidine propargyl ester.

The results show that estimation of the affinity constant by desensitisation consistently overestimated the value in comparison to estimation by receptor inactivation. This indicates that null methods of analysis cannot be applied to the desensitised data, possibly because desensitisation has also affected the stimulus-response coupling. Siegel et al., (1984) has shown that desensitisation of the ileal muscarinic receptor can be reversed by inhibitors of phospholipase A_2, indicating that desensitisation also affects arachidonic acid metabolism, in addition to changes in the receptor configuration (Young, 1972).

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THE Na-PARADOX: DETRIMENTAL CARDIAC EFFECTS DUE TO Na REPLETION IN ISOLATED RAT HEARTS.

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Readmission of Ca to hearts which have been perfused for a short period with Ca-free medium, precipitates severe myocardial damage and functional disturbances which has been attributed to the phenomenon called calciumparadox. The calcium-paradox attracted clinicians because Ca-free cardioplegic solutions had been used for myocardial protection in cardiac surgery. Deleterious effects upon reperfusion after cardioplegia as a result of the calciumparadox have been described. In addition, a number of cardioplegic solutions and non-ionic angiographic contrast solutions (iopamidol and iohexol) contain low or no amounts of Na. Little or no information is available about reperfusion with normal Na containing medium after a Na-poor perfusion period. The present study was performed to detect deleterious cardiac effects due to a possible sodium paradox phenomenon in analogy to the calciumparadox.

Isolated perfused rat hearts demonstrated a transient severe loss of contractile force after reperfusion with normal Na (256 mosmol/L); following a 30 minutes lasting low Na (50-100 mosmol/L ; equimolarly replaced by glucose and or mannitol) perfusion. Cardiac contractility restored after 5 minutes to a steady-state level significantly beneath control level. The Na reperfused hearts demonstrated ultrastructural damage which corresponded strongly with those find after the calcium-paradox: degenerated fibres with myolysis, round swollen mitochondria with electron dense aggregates and excessive contraction bands. However, neither dilated intercalated discs nor cleavage between glycocalyx and sarcolemma were seen. Also a characteristic massive leakage of creatinine phosphokinase as seen for the calciumparadox was absent during the Na reperfusion period. Pretreatment with the Na and/or Ca translocation interfering drugs like lidocaine (35-70 µM), verapamil (20-100 nM), ouabain (25-50 µM) and trifluoperazine (2.5-10 µM) could not prevent the transient strong decrease in myocardial contractility due to Na reperfusion. Except for ouabain these drugs potentiated the extent and the duration of the myocardial depression. Although ouabain did not prevent this decrease in contractility the restoration of contractility was significantly faster and more complete.

It is concluded that readmission of Na to hearts perfused with Na-poor solutions promotes cellular damage within the myocardium concomitantly with a transient decrease in myocardial contractility: the sodium paradox. Pharmacological screening could not delineate an underlying mechanism sofar although intracellular Ca derangements are anticipated. This finding might have serious implications for the clinical use of Na-poor cardioplegic and angiographic contrast solutions.

EFFECT OF N-ETHYLMALEIMIDE AND PERTUSSIS TOXIN ON THE MUSCARINIC-AGONIST RESPONSE IN RAT ATRIUM.

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It is well documented that stimulation of muscarinic(M)-receptors in the atrium in vitro leads to negative inotropic and chronotropic effects. Only the agonists which are vulnerable to acetylcholineesterase (AChE) are able to discriminate between the inotropic and chronotropic responses. Consequently this phenomenon can be abolished through the addition of an inhibitor of the enzyme AChE (Eglen et al. 1985). We investigated in more detail the M-agonist induced effects on rat atrium in vitro. Both right (spontaneously beating) and left (paced) atria were used. Experiments were performed at 37°C using a Muralt-Tyrode solution. In addition, radioligand binding experiments were performed using ^{3}H-N-methylscopolamine (0.4 nmol/l: spec.act. 81 Ci/mmol) as described by Doods et al. (1986).

In agreement with the findings of Eglen et al.(1985) none of the investigated agonists could discriminate between the negative inotropic and chronotropic effects after M-receptor stimulation when the atria were pre-incubated with an AChE inhibitor. In this respect diisopropylfluorophosphate was a more suitable compound compared to physostigmine, because the latter compound produced a direct negative inotropic effect. However, when we pre-incubated the atria with N-ethylmaleimide (NEM)(30 µmol/l) it was possible to discriminate between the negative inotropic and chronotropic responses after M-receptor stimulation. NEM blocked rapidly and time-dependently ($2\frac{1}{2}$, 5, 10 min) the negative chronotropic effect of carbachol, virtually without influencing the negative inotropic response. Similar experiments with the irreversible antagonist dibenamine showed that this effect of NEM is not the result of a difference in receptor-reserve for both responses. Radioligand binding experiments proved that the discriminating effect of NEM could not be explained by an interaction of NEM with nucleotide-sensitive coupling-proteins like Ni or No.

In atrial preparations obtained from rats which had been pre-treated with pertussis toxin, it was found that both the inotropic and the chronotropic effect after M-receptor stimulation were antagonized in a non-competitive manner. The inhibition of the response of carbachol was slightly more effective for the inotropic than for the negative chronotropic response.

We conclude:

1) The investigated agonists do not discriminate between the M-receptors responsible for the inotropy and chronotropy, respectively.
2) The ability of NEM to discriminate between the inotropic and chronotropic effects of carbachol after M-receptor stimulation may be a reflection of the existence of two different potassium channels in atrial tissue and SA-node, respectively, as suggested by Trautwein et al. (1981).
3) Both the inotropic and the chronotropic responses observed after M-receptor stimulation are coupled to the receptor via pertussis toxin sensitive coupling (N) proteins.

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BOTH GLUCOCORTICOIDS AND CYCLIC AMP PROCESSES AFFECT ACh STORES IN RAT DIAPHRAGM PREPARATIONS, IN VITRO.

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Glucocorticoids have direct effects on neuromuscular transmission (1). Dexamethasone (Dex, 0.2 µM) increases the rate of radioactive choline (Ch) uptake and its incorporation into acetylcholine (ACh) in the endplate-rich area of rat phrenic nerve-hemidiaphragm preparations, in both indirectly-stimulated and resting tissue (1). Using autoradiography, it was shown that Dex (0.2 µM) increases Ch uptake in phrenic nerve terminals but not in muscle fibres (2). Dex (0.2 µM) increases both the resting and evoked output and turnover of ACh in vitro, without altering the total amount of ACh in the tissue (1). At higher concentrations (> 0.6 µM), Dex inhibits Ch uptake in the endplate-rich area of stimulated hemidiaphragms, although there is no effect on tissue ACh content. With 2 µM Dex the ACh content is increased to 140% of the control value. No increase in ACh is found at higher Dex concentrations (> 6 µM). This biphasic Dex concentration-effect curve is also found in the presence of the anticholinesterase physostigmine (Physo, 15 µM). In this case, greater amounts of tissue ACh are found over a wider concentration range (2 to 25 µM Dex). This increase in ACh is specific for glucocorticoids. No increase in ACh is found in the presence of the nicotinic receptor antagonists d-tubocurarine (5 µM) or alpha-cobrotoxin (5 µg/ml). The glucocorticoid-specific increase in ACh stores may perhaps be caused by some interaction of the corticosteroids with presynaptic ACh receptors regulating ACh synthesis and/or output. Wilson (3) suggested that cyclic AMP processes were involved in regulating ACh synthesis, storage and output. Hemidiaphragm ACh content can be increased by dibutyryl-cAMP (10 µM). This increase occurs in the presence of Physo (15 µM) and is abolished by d-tubocurarine (5 µM). It is also found in the presence of 0.2 µM Dex, although it is abolished by 2 µM Dex. It is tentatively suggested that in phrenic nerve endings, both Dex and cAMP processes may be interacting with a presynaptic ACh receptor.

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EVIDENCE FOR PRESYNAPTIC MUSCARINIC RECEPTORS INVOLVED IN THE EFFECTS OF F7-TOXIN ON RODENT MOTOR ENDPLATE.

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Muscarinic presynaptic receptors have been described in connection with postsynaptic muscarinic receptors in vertebrates, and in connection with nicotinic receptors in invertebrates.

In the rat phrenic nerve-diaphragm preparation the toxin F7, isolated from the venom of the green mamba Dendroaspis angusticeps, has some presynaptic effects that can be antagonized by atropine. Spontaneous fasciculations are seen in mice 20 min after injection (i.p.) of 5 µg/g F7-toxin in vivo and after superfusion of rat diaphragm in vitro with 0.5-5 µg/ml F7-toxin. The spontaneous firing rate of the miniature endplate potentials (mepps) is increased at the same time and mepps become clustered in bursts or are superimposed as giant mepps. The spontaneous fasciculations could be depressed by (+)-tubocurarine, 2 µmol/l, but the clustering of mepps and the presence of giant mepps is not antagonized. Atropine (1-5 µmol/l) does not diminish the spontaneous firing rate of the mepps. The clustering of the mepps and the presence of giant mepps, however, is strongly depressed by atropine in a dose dependent way.

It is known that atropine, in a rather high concentration of 10 µmol/l, has a facilitatory effect on ACh release only in the presence of physostigmine. Lee et al (1984) have shown that F7-toxin displays potent anticholinesterase activity.

We found that the rising and decay times of some miniature endplate currents were both prolonged and/or normal in the same registration. Neither postsynaptic effects nor anticholinesterase activity can fully explain the observed effects of F7-toxin.

We tentatively conclude that the effect of F7-toxin on the ACh concentration can be blocked at the postsynaptic site by (+)-tubocurarine and that at least some of the presynaptic effects of F7-toxin are mediated by presynaptic muscarinic receptors which, can be antagonized by atropine.

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THE RESPONSES TO ISOPRENALINE IN THE ELECTRICALLY STIMULATED GUINEA-PIG ILEUM ARE NOT COMPETITIVELY ANTAGONIZED BY PROPRANOLOL.

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Alpha$_2$-adrenoceptor agonists of the phenylethylamine (PEA) class can be distinguished from agonists of the imidazoli(di)ne group by antagonists. For instance, in the field stimulated guinea pig ileum and rat vas deferens clonidine always interacts competitively with alpha$_2$-adrenoceptor antagonists (e.g.: yohimbine, rauwolscine, idazoxan) whereas noradrenaline and alpha-methylnoradrenaline often deviate from true competitive antagonism (Mottram 1983; Hicks et al, 1985; Bond et al, 1986). Further evidence for differences between the imidazoli(di)ne and PEA agonists derive from experiments in guinea pig ileum using the irreversible alpha-adrenoceptor antagonist, benextramine. Benextramine, at a concentration which totally abolishes responses to clonidine, spares responses to noradrenaline; a difference which cannot be attributed to alpha$_2$-adrenoceptor reserve (Bond et al, 1986).

One hypothesis advanced to explain these discrepancies is the possibility that PEA agonists evoke activity at a site distinct from the receptor shared by the imidazoli(di)ne agonists and the alpha$_2$-antagonists (Bond et al, 1986). Although investigators have used propranolol (usually 1 μM) to block beta-adrenoceptors it remains possible that the much greater affinity and activity of the PEA's for beta-adrenoceptors could produce the above differences by breaking through the propranolol induced beta-blockade. Thus, the present experiments were undertaken to examine this possibility using the electrically field stimulated guinea pig ileum with neuronal and extraneuronal uptake inhibited as described previously (Bond et al, 1986). All experiments were done using the potent beta-adrenoceptor agonist (-)-isoprenaline (IPNE).

IPNE produced a biphasic inhibition of the cholinergically evoked 'twitch response'. The first phase caused a 30-40% inhibition with an IC_{50} of 30 nM. The second phase (70-100% inhibition) required much higher concentrations of IPNE (beyond 3 μM) and was inhibited completely by the alpha$_2$-adrenoceptor antagonist, idazoxan (3 μM). Thus, the second phase was attributed to alpha$_2$-adrenoceptor activation by IPNE. All further studies were done in the presence of idazoxan (3 μM). Table 1 shows the lack of competitive antagonism toward IPNE with propranolol when used over a 50 fold concentration range.

Table 1. Effect of propranolol and nadolol on inhibitory responses to IPNE in the guinea pig ileum.

Antagonist	Concentration (μM)	Concentration-Ratios of IPNE
None (time control)	0	1.1
Propranolol	0.1	8.5 ± 3
Propranolol	1	10.2 ± 3
Propranolol	5	14.7 ± 2
Nadolol	10	8.0 ± 3

None of the antagonist induced concentration-ratios differ significantly $P \leq 0.05$

The results reveal an inhibitory response to IPNE which is resistant to blockade by the combination of idazoxan and propranolol and idazoxan and nadolol. Because inhibitory alpha$_1$- adrenoceptors are absent from the guinea pig ileum, the resistant responses to IPNE appear to result from a site unrelated to currently defined alpha and beta-adrenoceptors. This site may be the actual reason for the differences between PEA and imidazolidine agonists, rather than inadequate beta-adrenoceptor blockade.

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DECREASE IN α_1-ADRENOCEPTORS OF CHICK SMOOTH MUSCLE AFTER IRREVERSIBLE SYMPATHETIC DENERVATION.

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The expansor secundariorum (ESM), a discrete smooth muscle in the chick wing, contains α_1-adrenoceptors which mediate contraction of the muscle and which can be selectively labelled with [^{3}H]-prazosin (Bennett et al., 1986a). We have previously reported that the α_1-adrenoceptor number in the ESM is decreased after reversible noradrenergic denervation with 6-hydroxydopamine (6OHDA) (Bennett et al., 1986b). We have now examined the effects of irreversible surgical denervation on α_1-adrenoceptor number and contractile responsiveness of the ESM.

The left ESM was denervated as previously described (Bennett et al., 1982) and the nerve supply to the right ESM left intact to serve as a control. Chicks were killed 7 days after denervation and the ESMs removed. ESMs from 2 chicks were pooled for ligand-binding studies, which were performed as previously described (Bennett et al., 1986b) at a single concentration of [^{3}H]-prazosin (1.2nM). Tissue catecholamines were extracted by a modification of the method of Anton & Sayre (1962) and noradrenaline (NA) levels measured by HPLC with electrochemical detection. ESMs were mounted in 20ml organ baths as described (Bennett et al., 1982); non-cumulative dose-response curves to methoxamine (10^{-7} to 3×10^{-5}M) were obtained and maximum response (R_{max}) and EC_{50} values calculated. Results are summarized in Table 1.

Table 1 Effects of surgical denervation on the ESM (means ± s.e.mean)

	Control	Denervated	n
Specific [^{3}H]-prazosin bound			
(fmols/mg protein)	17.3 ± 0.9	8.2 ± 0.7 **b	9
(fmols/mg wet weight)	1.91± 0.11	1.03± 0.08**b	9
NA (pmols/mg wet weight)	7.10± 0.75	0.32± 0.05**a	8
Methoxamine -log EC_{50}(M)	5.73± 0.02	5.88± 0.03*a	10
Methoxamine R_{max} (g/g wet weight)	101.3 ±18.6	59.9 ± 5.5	10

* $p<0.002$, ** $p<0.001$ by Student's paired (a) or unpaired (b) t-test

Thus surgical denervation of the ESM produced a 50% decrease in apparent α_1-adrenoceptor number, similar to the effect we previously described after chemical denervation with 6OHDA (Bennett et al., 1986b). Maximum contractile response to α_1-adrenoceptor stimulation was apparently, but not significantly, reduced, whilst sensitivity was increased after denervation. The latter effect may have been due to development of a non-specific supersensitivity, often observed after sympathetic denervation (see Fleming et al., 1973).

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EFFECTS OF SIX DRUGS ON THE CONDUCTION PROPERTIES OF ISOLATED FROG SCIATIC NERVES.

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The effects of six representative drugs on the conduction properties of isolated frog sciatic nerves have been studied. These are: a local anaesthetic agent (lidocaine), an antimuscarinic agent (atropine), a benxodiazepine compound and also antianxiety agent (diazepam), a CNS stimulant (strychnine), an antimalarial, antiarrythmic agent (quinidine) and an anticholinesterase (eserine).

Isolated frog sciatic nerves dipped in the drug solutions ranging in concentrations from a tenth of a mM to a tenth of a uM were examined for their conduction properties with the help of a setup, the final component of which was a cathode ray oscilloscope. These studies reveal that all the six drugs have somewhat similar effects on the action potential profiles, with progressive changes in the conduction parameters such as the maximum amplitude, duration and detection period. Comparison of the results obtained in the present studies with those obtained earlier on the effects of Co-60 gamma radiations (Nayar & Srinivasan, 1975a,b) and UV radiations (Tripathy & Srinivasan, 1982) reveal that the drugs, in contrast to radiations, appear to influence to different extents the maximum amplitude and the area under the positive peak of the action potential profile, suggesting that the effects due to drugs are more complicated in nature than those due to radiations.

A rigorous quantitative examination of the action potential profiles was done with the help of a computer. Making use of a mathematical expression, it has been found possible to computer-simulate the experimentally obtained action potential profile. Additionally, new parameters were obtained which were found to have biological significance.

The effects of the drugs on the biological activities of the enzyme AChE from bovine erythrocytes as well as the true and pseudo ChE from human RBC and serum were studied. Except eserine none of the other drugs had any effect on the activity of the enzyme. Gel electrophoretic analyses confirmed these observations.

Ultraviolet absorption spectral as well as ultraviolet different absorption spectral measurements of the enzyme complexed with the drugs clearly point to changes in the conformation of the enzyme concomitant with the complexing with the drug. The changes were however different in nature than those obtained with the gamma irradiated enzyme, where tryptophan and tyrosine groups were found to get exposed (Nayar & Srinivasan, 1975c).

The present studies examined against the background of the data previously available clearly indicate that the effects of drugs on the nerves are more complicated in nature and invite more investigations.

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EFFECT OF QUINIDINE AND SOME LOCAL ANAESTHETICS ON CONTRACTILE RESPONSES OF RAT ANOCOCCYGEUS MUSCLE.

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Quinidine shares potent local anaesthetic property with procaine, lignocaine and amethocaine. In addition, the compound has been shown to possess anti-adrenergic and anticholinergic actions. The present investigations were to examine the four compounds against electrically - and drug-induced contractions of the isolated anococcygeus since local anaesthetic in general are known to cause disruption of Ca^{2+} dependent physiological functions (Feinstein et al., 1968). The anococcygeus muscle was isolated according to Gillespie (1972) and suspended under a resting tension of 0.75 g in Tyrode solution (gassed with air) at 37°C. Contractions were recorded isometrically through Grass FT 03C force-displacement transducer coupled to Grass polygraph model 7D.

Quinidine (10^{-7} - 10^{-5}M) caused a non-competitive antagonism of noradrenaline, (NA) carbachol and transmurally (supramaximal voltage, 1ms, 200 pulses) evoked contractions. Concentrations of quinidine below 10^{-7}M did not inhibit transmural stimulations. Contractions induced to $CaCl_2$ in tissues bathed with Ca^{2+}-free depolarizing Tyrode solution were also antagonized (10^{-8} - 10^{-6}M). These observation suggests that quinidine action is non-specific in nature. Classical local anaesthetic agents, procaine, lignocaine and amethocaine did not inhibit NA even though they antagonized responses to transmural stimulation, carbachol and $CaCl_2$. Infact procaine (10^{-5} - 10^{-4}M) caused remarkable enhancement of NA contractions. Since of the four compounds, only quinidine exhibited post-synaptic inhibition against NA, the observation could be taken to confirm the antiadrenergic (Motulsky et al., 1984) property of the compound. However, previous occupation of the α-adrenoceptor sites with a high concentration (5.0×10^{-5}M) of NA protected against phenoxybenzamine but not against quinidine block of NA contractions. This suggests that the antiadrenergic action of quinidine was not through α-receptor blockade but through an intracellular event possibly associated with the receptors. The ineffectiveness of the local anaesthetics against post-synaptic actions of NA is not understood. Perhaps, they exerted effects similar to the calcium entry blockers which are incapable of blocking Ca^{2+} channels mobilized for contractions by NA (Oriowo, 1982).

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THYMUS REGENERATION IN OLD RATS AFTER ORCHIDECTOMY: EFFECTS OF VARIOUS STEROIDS.

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The thymus is critically important during development, but atrophies progressively after puberty, and is generally considered to play no part in the immune system of the adult. Recently, we have shown (Fitzpatrick et al, 1985) that the thymus, which has apparently disappeared in 18 month old rats, is regenerated by one month after orchidectomy. In these animals, thymus regeneration is inhibited by testosterone (Adcock et al, 1986) and in the present communication we show the results of experiments aimed at studying the hormonal specificity of the inhibitory effect.

Male rats of 12 to 15 months, weighing 500 to 600g, were bilaterally orchidectomized (Sagatal: May & Baker: 28mg/kg i.p.). On the 7th day after orchidectomy, rats were given S.C. implants in 'Silastic' tubing of 25mg of one of either testosterone, oestradiol, progesterone, corticosterone or 5 alpha-dihydrotestosterone (DHT) under ether anaesthesia. One group received an empty implant. On day 30, the rats were anaesthetised with ether and bled by cardiac puncture. The thymus, prostate and seminal vesicle glands and the spleen were removed, weighed and retained for histology. A count of total white cells in circulating blood was made.

Sham-implanted rats had large multilobular thymuses which were well-defined histologically, and significantly larger than those from all the steroid-treated animals except in the case of corticosterone (Table 1). Testosterone and oestradiol were equipotent in producing the greatest loss of thymus weight. DHT, which is a potent androgenic metabolite of testosterone, significantly reduced the size of the thymus, but its effects appeared to be variable, and the thymus was more lobular and densely packed with thymocytes than thymuses from testosterone or oestradiol-treated rats. The pattern of suppression of thymus weights was similar to the pattern of the total white cell counts for the different treatment groups (Table 1). This suggests that the regenerated thymus was contributing to the pool of circulating white cells.

Table 1 Effects of orchidectomy and steroid treatment on thymus weight and total white cell count (mean ± s.e. mean)

steroid	thymus (mg/g body weight)	white cell/l x 10^{-6}
---	6.03 ± 0.3	7.85 ± 1.1 (4)
testosterone	3.04 ± 0.5	3.41 ± 0.6 (4)
oestradiol	3.15 ± 0.5	4.32 ± 0.8 (5)
DHT	4.07 ± 0.5	5.67 ± 1.0 (5)
corticosterone	4.55 ± 0.7	3.55 ± 0.4 (5)
progesterone	4.61 ± 0.2	6.27 ± 0.4 (5)

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CAPSAICIN-SENSITIVE AFFERENT NEURONES INVOLVED IN GASTRIC MUCOSAL PROTECTION.

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Functional ablation of afferent neurones by systemic treatment of rats with capsaicin has previously been shown to aggravate gastric ulceration in response to pylorus ligation and acid distension of the stomach (Szolcsányi & Barthó, 1981). The present study attempted to further elucidate a possible role of capsaicin-sensitive afferent neurones in gastric mucosal protection.

Gastric ulceration was induced by indomethacin (14-42 μmol kg^{-1} s.c.) or ethanol (15-25 %, 3 ml kg^{-1} intragastrically). It was found that, in adult rats treated with a high dose of capsaicin (160 μmol kg^{-1} s.c.) as neonates, the formation of gastric mucosal lesions was greatly enhanced as compared with vehicle-treated controls. This aggravation of gastric ulceration was not due to a deficit of prostaglandins E_2 and I_2 in gastric tissue. Experiments involving atropine, hexamethonium, guanethidine, cimetidine, and terbutaline indicated that the enhancement of gastric ulceration seen in capsaicin-treated rats did not result from changes in the function of the autonomic nervous system or of gastric histamine H_2 receptors and ß-adrenoceptors. It would therefore appear that the aggravation of gastric ulceration is caused by the ablation of afferent neurones, and that capsaicin-sensitive afferent neurones participate in mechanisms which protect the gastric mucosa against ulcer formation.

This sensory nerve-mediated protection of the gastric mucosa can apparently be activated by intragastric administration of low doses of capsaicin (0.5-2 μmol kg^{-1}). Such low doses of capsaicin were found to reduce gastric ulceration induced by ethanol (25 %, 3 ml kg^{-1} intragastrically). This protective effect of capsaicin was independent of prostaglandin formation since it was not affected by indomethacin. Furthermore, the protective effect of capsaicin remained unaltered following subdiaphragmatic vagotomy, surgical removal of the coeliac ganglion, and pretreatment of the rats with atropine or guanethidine. This indicated that gastric mucosal protection induced by capsaicin did not involve the autonomic nervous system. However, the protective effect of low doses of capsaicin depended on the integrity of sensory neurones, since it was absent in rats which had been treated with a high dose of capsaicin as neonates.

These observations provide further evidence that gastric mucosal protection can in part be achieved by stimulation of capsaicin-sensitive afferent neurones. Because capsaicin is known to increase mucosal blood flow in the rat stomach (Limlomwongse et al., 1979) it may be hypothesized that sensory nerve-mediated gastric mucosal protection is due to release of vasodilator or otherwise protective factors (neuropeptides) from sensory nerve endings in the gastric mucosa.

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DETERMINATION OF PLATELET GABA-TRANSAMINASE (GABA-T) ACTIVITY AS A MEANS OF ASSESSING THE EFFECT OF GABA-T INHIBITORS.

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Since GABA was recognised as a major inhibitory neurotransmitter in the central nervous system (Roberts, et al. 1976; Curtis, 1979), several theories have been put forward suggesting deficiencies in GABA-ergic neuronal function as a possible cause of epilepsy (Meldrum 1978; Spero 1982). Administration of vigabatrin, an irreversible GABA-T inhibitor, to patients has produced a significant increase in cerebrospinal fluid (CSF) GABA concentration (Grove et al. 1980) and an encouraging clinical improvement in epilepsy (Rimmer & Richens 1984).

Human blood platelets have substantial GABA-T activity (White 1979) and measurement of platelet GABA-T activity may be a convenient method of assessing drug effects in patients on GABA-T inhibitors in whom CSF sampling is not justified.

Four healthy male volunteers (27-47 years old) were each given vigabatrin 3g as a single oral dose. Ten ml blood samples were withdrawn from each volunteer at various intervals from day 1 of drug treatment to day 10. Platelets were separated, washed, lysed and assayed for GABA-T activity, according to the method of White (1979), and for protein concentration, according to the method described by Bradford (1976). Plasma vigabatrin was assayed by HPLC following dansylation using the method of Merrell Pharmaceuticals Ltd. (personal communication).

There was a good correlation between absorbance and protein concentration ($r=0.998$) over the range 100 to 1000 µg/ml. The GABA-T activity before drug administration was 40 ± 4.8 pmol/min/mg protein (mean ± SD). Enzyme activity was inhibited by 55% ($P<0.01$, $t = 6.75$) two hours after drug administration and the inhibition was 30% ($P<0.05$, $t = 2.66$) 48 hours later. A return to full enzyme activity ($P>0.05$, $t = 0.84$) was observed in samples obtained 96 hours following drug administration. The initial rise in plasma vigabatrin concentration coincided with the enzyme inhibition.

These preliminary results suggest that measurement of platelet GABA-T activity may be a useful method of monitoring the effect of GABA-T inhibitors.

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HUMAN PLATELET β_2-ADRENOCEPTORS UNDERLIE SIMILAR REGULATORY MECHANISMS AS LYMPHOCYTE β_2-ADRENOCEPTORS.

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Human lymphocytes containing a homogeneous population of β_2-adrenoceptors (R) are a frequently used model to study changes in β-R in man.Thus, it has been demonstrated that longterm treatment of volunteers with the non-selective β-R antagonist propranolol (no intrinsic sympathomimetic activity (ISA)) leads to an about 35% increase in lymphocyte β_2-R density, whereas longterm treatment with pindolol (non-selective, strong ISA) decreases lymphocyte β_2-R density by about 50% (Brinkmann et al., 1985). Furthermore, acute stimulation of sympathetic activity by dynamic exercise on a bicycle leads to a rapid 100% increase in β_2-R density (Brodde et al., 1984).

Recently it was shown, that in addition to α_2-R human platelets contain a homogeneous population of β_2-R of low capacity that are weakly coupled to the adenylate cyclase (Cook et al., 1985; Wang and Brodde, 1985).However, up to now nothing is known about the regulation of human platelet β_2-R. Therefore, we have investigated the effects of propranolol and pindolol as well as of dynamic exercise on human platelet β_2-R density.

24 healthy normotensive volunteers aged 25.4±2.4(19-35) years participated in the study after having given informed written consent. Propranolol (4x40 mg/d) and pindolol (2x5 mg/d) were administered orally for one week. The dynamic exercise test was performed on a bicycle in supine position for 15 minutes at a maximal work load of 125-150 W as described recently (Brodde et al., 1984). Platelet membranes were prepared from 80 ml EDTA-blood and platelet β_2-R density was determined by (-)-^{125}I-iodopindolol (IPIN) binding at 6-8 concentrations ranging from 10-200 pM (Wang and Brodde, 1985).

Propranolol increased platelet β_2-R density by about 40%; after withdrawal β_2-R density declined slowly and was still elevated for 3 days. On the other hand, pindolol decreased platelet β_2-R density by about 50%; after withdrawal β_2-R density recovered very slowly still being significantly reduced after 4 days.

Exercise caused an increase in systolic blood pressure from 123.7± 4.1 to 187±4.2 mm Hg in 10 healthy volunteers; this was accompanied by about 2-3 fold increases in plasma catecholamine levels. At the end of exercise platelet β_2-R density had significantly increased from 3.64±0.3 to 5.13±0.35 fmol IPIN bound/mg protein. One hour after exercise all parameters had returned to values similar to or slightly below pre-exercise values.

These results demonstrate that platelet β_2-R underlie regulatory mechanisms which are very similar to those of lymphocyte β_2-R. Therefore, human platelets possessing both α_2-R and β_2-R are a suitable model to study α- and β-R interactions in man.

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CHARACTERISATION OF A 5-HYDROXYTRYPTAMINE RECEPTOR IN THE FOREGUT OF THE LOCUST SCHISTOCERCA GREGARIA.

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Freeman (1966) and Huddart & Oldfield (1982) demonstrated that the foregut of the locust Locusta migratoria was sensitive to 5-HT (10^{-8} - 10^{-5} M) showing an increase in tone as well as an increase in the amplitude and frequency of spontaneous contractions. The foregut of the locust Schistocerca gregaria is innervated by nerves originating in the stomatogastric nervous system, via the suboesophageal and ingluvial ganglia; and the corpora cardiaca, where 5-HT is known to be present (Klemm, 1972). The aim of this study was to investigate the effect of 5-HT on the isolated foregut of Schistocerca gregaria and to attempt a classification of the 5-HT receptor mediating this effect.

Isolated foreguts (oesophagus to proventriculus) of Schistocerca gregaria were incubated in Clarke Insect Ringer at room temperature (18 ± 2°C) for 20 min prior to testing the effects of 5-HT and a range of other compounds using a 6 min dose cycle with 2 washes. Dose response curves were constructed for 5-HT, 5,6-dihydroxytryptamine (5,6-DHT), MK212 (6-chloro-2-(1-piperazinyl)pyrazine) and proctolin while the tissue was also tested for responses to glutamate, ACh, dopamine (DA) and octopamine (OA). The effect of mianserin (10^{-7} M - 5 x 10^{-5} M) on the 5-HT response was investigated, the tissue being incubated with the antagonist for 20 min prior to testing the effects of 5-HT.

The tissue showed no spontaneous contractile activity but 5-HT (10^{-10} M - 10^{-8} M) caused an increase in the tone of some tissues. 5-HT (2 x 10^{-8} M - 3 x 10^{-6} M; ED_{50} : 4 ± 0.6 x 10^{-7} M; n = 18) caused dose dependent relaxation of the gut showing marked tachyphylaxis at 10^{-5} M. Proctolin, (10^{-9} M - 10^{-6} M; ED_{50} : 1.6 ± 0.3 x 10^{-7} M; n = 6) caused dose dependent contraction of the foregut and consequently the general tonus of the tissue was maintained using alternate doses of proctolin (10^{-7} M) and 5-HT. MK212 (10^{-7} M - 5 x 10^{-6} M; ED_{50} : 1.2 ± 0.2 x 10^{-6} M; n = 6) and 5,6-DHT (10^{-6} M - 5 x 10^{-4} M; ED_{50} : 9 ± 0.2 x 10^{-5} M; n = 6) also caused dose dependent relaxation of the foregut, but their efficacy was much less than that of 5-HT. OA and DA (both 2 x 10^{-7} M - 2 x 10^{-5} M) also caused relaxation but the response to the latter was very weak while ACh (10^{-9} M - 10^{-4} M) had no effect on the tissue. By comparison glutamate (10^{-8} M - 10^{-6} M) caused weak dose dependent contraction of the tissue. Mianserin (10^{-7} M - 5 x 10^{-5} M; n = 8) was found to be a competitive antagonist of 5-HT (pA_2 = 6.3 slope = 0.78) with the 5-HT dose response curves being displaced to the right in a dose dependent manner. Furthermore, mianserin also caused weak, dose-dependent contraction of the tissue at this range of concentrations.

The 5-HT-induced relaxation of the foregut of Schistocerca gregaria is an opposite effect to that observed in Locusta migratoria (Freeman, 1966; Huddart & Oldfield, 1982). The agonist effect of 5,6-DHT was also observed by Colhoun (1963) on the foregut of Periplaneta americana suggesting that 5,6-DHT may be a 5-HT agonist in the periphery of insects. Antagonism of 5-HT induced relaxation by mianserin and the dose dependent relaxation caused by the 5-HT_2 agonist MK212 suggests that this 5-HT receptor has properties in common with mammalian 5-HT_2 receptors.

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CONCENTRATION-ANTICONVULSANT EFFECT RELATIONSHIPS OF PHENOBARBITAL IN RATS.

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Investigation of factors influencing drug concentration-pharmacological effect relationships requires the establishment of experimental strategies that can clearly distinct between alterations in pharmacokinetics and pharmacodynamics. Phenobarbital (PB) has been proven to possess favourable characteristics for this kind of studies, viz. it is not optically active and probably not biotransformed to (inter)active metabolites (1). To circumvent potential complications due to distribution disequilibrium between different body compartments, a study was initiated in pseudo steady-state. One hour after single i.v. bolus doses constant concentration ratios between serum, brain and cerebrospinal fluid (CSF) were obtained. A range of concentrations in these compartments, attained after i.v. bolus doses (0-75 mg/kg) and predicted on the basis of previously determined pharmacokinetic parameters, were correlated to the anticonvulsant activity of the drug. This effect was determined as the pentylenetetrazol (PTZ) concentration threshold in serum required to elicit a first myoclonic twitch during a slow i.v. infusion of PTZ. The anticonvulsant response was expressed as an elevation of the baseline threshold (no pretreatment with PB) determined 48 hrs earlier. By using every rat as its own control interindividual variability was reduced. At the onset of effect CSF, serum and total forebrain were sampled successively. PTZ was analysed by GLC and PB by HPLC. Concentrations of free PB in serum were determined by equilibrium dialysis.

The empirical concentration-effect relationships were, on theoretical grounds, fitted to the sigmoid Emax model (Hill equation) by the non-linear least squares computerprogram ELSMOS. The shape of the obtained curves was similar for the different sampled compartments. The various pharmacodynamic parameters (Mean ± SEM), as determined by the best fit, are presented in the table:

	EC50 PB	Emax	n
	µg/ml,µg/g	PTZ µg/ml	
serum (total)	92.1 ± 8.3	121.3 ± 15.9	3.25
serum (free)	51.9 ± 6.0	115.1 ± 16.0	2.80
CSF	50.0 ± 7.3	106.8 ± 21.8	3.01
brain	63.7 ± 8.4	127.7 ± 27.7	3.28

Statistical evaluation of these parameters demonstrates the reliability of the established concentration-effect relationships: the Emax values do not differ significantly and the EC50 values closely correspond with the protein binding (free fraction 60%) and the concentration ratio brain/serum (0.6) of PB.
It can be concluded that this experimental strategy offers the possibility to study factors that influence the concentration-anticonvulsant effect relationship of PB.

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INDOMETHACIN, D-PENICILLAMINE AND DEXAMETHASONE ON CARTILAGE BREAKDOWN BY A GRANULOMATOUS REACTION.

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Irreversible destruction of the connective tissues of articulating joints is the most serious consequence of the chronic inflammatory disease, rheumatoid arthritis (RA). Histopathological studies show much of this to be caused by an invasive granulomatous tissue called pannus that is derived from the synovial membrane and its microvasculature. Recently we (de Brito and Willoughby, 1985; de Brito et al, 1986) and others (Bottomley et al, 1986) demonstrated the susceptibility of cartilage to damage by a granulomatous reaction induced by cotton in subcutaneous (sc) tissues of rodents. We also suggested its usefullness as a model of connective tissue destruction in chronic inflammatory disorders. We have therefore undertaken an investigation to determine how this model is affected by drugs. This communication presents our preliminary findings with three types of drugs used in the treatment of RA.

Freshly collected intact Wistar rat (150-200 gm) femoral head cartilage was implanted alone or wrapped with 5 mg sterile cotton into the sc tissues of the flanks of ether anaesthetised Balb c inbred mice (20-30 gm). Begining one day after implantation groups of 6-8 mice with cotton-cartilage implants were dosed orally with drug or vehicle (tap water) daily. Two weeks later all mice were exsanguinated and the implants were removed. The granulomatous reaction induced by cotton was assessed by determining its content of water (wet weight - dry weight of implanted cotton) and granulation tissue (dry weight - original weight of cotton). Cartilage proteoglycan was determined by the method of Farndale et al (1982) and serum haptoglobin by single radial immunodiffusion.

Table

Implant/Drug		Cotton Granulomatous Response		Cartilage	Serum
		Water content	Granulation tissue	Proteoglycan	Haptoglobin
		mg	mg	µg	mm
Cartilage(100%)		0	0	444 ± 16	1.15 ± 0.50
Cotton-Cartilage(0%)		47.0 ± 2.3	7.57 ± 0.47	189 ± 16	8.94 ± 0.95
		% Inhibition of cotton induced response by drug (mg/kg)			
Indomethacin	1	27**	32*	0	-24
D-Penicillamine	100	5	7	2	38
	200	-3	-8	-9	21
Dexamethasone	0.01	-1	15	-5	0
	0.03	33*	42*	37*	32

* $p<0.05$; ** $p<0.01$ significance levels (Student's t-test) determined on original results.

Of the three drugs tested, only dexamethasone at a high dose level inhibited loss of the cartilage matrix component proteoglycan. Significant partial suppression of the cotton granulomatous response was achieved with both dexamethasone and indomethacin but again only with maximally tolerated doses. D-Penicillamine was without effect on either parameter. However both D-Penicillamine and dexamethasone appeared to lower circulating levels of the acute phase protein, haptoglobin, but statistical significance was not reached. Further investigations on the effects of drugs in this model are currently in progress.

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β-ADRENOCEPTOR AGONISTS INFLUENCE H_2O_2 RELEASE OF GUINEA PIG ALVEOLAR MACROPHAGES.

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Alveolar macrophages (aMϕ) are the primary defenders in the lung against inhaled particles. These cells exhibit a variety of biological activities, like phagocytosis and killing of microorganisms and secretion of enzymes, reactive oxygen metabolites, prostaglandins, leukotrienes and other mediators. The function of these cells are under the influence of a great number of physiological and environmental factors. There are some indications that Mϕ possess β-adrenoceptors and that the activity of Mϕ is regulated by cyclic-AMP levels in the cell. In our study, we investigated the effects of a number of β-adrenoceptor stimulants on the phagocytic and metabolic activity of guinea pig aMϕ.

The aMϕ were obtained by lavage of the lungs of male guinea pigs. The cell suspensions were washed, differentiated and brought to a concentration of $5x10^6$ cells/ml. To quantitate phagocytic cell activity, radiolabeled, preopsonized bacteria were used (Henricks et al., 1983). Hydrogen peroxide (H_2O_2) production was determined using the horseradish peroxidase-mediated oxidation of phenol red by H_2O_2 (Pick & Keisari, 1980).

When $2.5x10^6$ aMϕ were stimulated with 25 ng phorbol myristate acetate (PMA), the aMϕ produced 10.5±0.5 nmol H_2O_2/5 min. In the presence of increasing amounts of adrenaline, noradrenaline, isoprenaline and dobutamine, release of H_2O_2 by PMA-stimulated aMϕ was dose-dependently diminished (see Table 1). In contrast, the β_2-agonist salbutamol had no effect on the H_2O_2 release of aMϕ (see Table 1).

Table 1 %H_2O_2 released by PMA-stimulated aMϕ in the presence of β-adrenoceptor agonists compared to control values.

concentration	adrenaline	noradrenaline	isoprenaline	dobutamine	salbutamol
$5x10^{-7}$ M	108±3	110±9	99±3	94±3	n.d.
10^{-6} M	92±2	93±4	91±4	86±7	103±3
$2.5x10^{-6}$ M	79±4	73±6	65±13	78±5	n.d.
$5x10^{-6}$ M	58±7	44±3	47±10	53±3	n.d.
10^{-5} M	30±5	20±5	22±5	23±4	98±4
$2x10^{-5}$ M	18±4	16±4	4±3	8±3	105±9

Beside H_2O_2 release, the uptake of bacteria by aMϕ was determined. When 20 bacteria/aMϕ were added and incubated for 5 min, 7±2 bacteria were phagocytized by the aMϕ. Addition of $2x10^{-5}$ isoprenaline, dobutamine or salbutamol to the incubation mixture had no effect on the phagocytic capacity of the aMϕ.

The decreased release of H_2O_2 by stimulated aMϕ in the presence of β-agonists could be caused by increased cyclic-AMP levels intracellularly due to stimulation of β-adrenoceptors on the membrane of the aMϕ. The fact that salbutamol had no effect on the H_2O_2 release seems to point to a β_1-adrenoceptor-mediated phenomenon. However, further studies are needed to elucidate the exact nature of the receptor. The β-agonists had no effect on the phagocytic activity of the aMϕ. In a previous study, we also observed no effects of cyclic-AMP-inducing agents on phagocytosis of polymorphonuclear leucocytes (Henricks et al., 1984).

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HISTAMINE RELEASE FROM GUINEA-PIG, HUMAN, RABBIT AND RAT MAST CELLS ELICITED BY CREMOPHOR EL AND ITS DERIVATIVES.

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Cremophor El is commonly used in the pharmaceutical industry to aid the solubilization of water-insoluble drugs. Although in man, this agent does not produce histamine release when administered alone, in the presence of other drugs it has been implicated in causing such reactions. We have studied the action of Cremophor El and its derivatives using an anaesthetised dog model and found that those derivatives based on 12-hydroxystearic acid (12-HSA) produced the lowest incidence of histamine release (Lorenz et al, 1977). In order to examine the mechanism of this histamine release more closely, we have investigated these substances on isolated mast cells from guinea-pigs, humans, rabbits and rats.

Solutions containing mast cells from lungs of the guinea-pig, human and rabbit were obtained by incubation of the tissues with collagenase (Ennis 1982) and rat peritoneal mast cells were obtained by peritoneal lavage. The cell suspensions were prewarmed for 5 min and then Cremophor El or one of the derivatives of 12-HSA was added to final concentrations between 100-0.1 μg/ml. The cells were then incubated for a further 10 min and the reactions stopped by the addition of ice cold buffer and centrifugation. Cremophor EL and the derivatives of 12-HSA were supplied by BASF (Ludwigshafen, F.R.G.).Those tested on the isolated cells were: TN (12-HSA polymerized with ethylene oxide; degree of polymerization 15; 15 EO), DH (diester of 12-HSA esterified with polyethylene glycol 600; PEG) and ME (monoester of 12-HSA esterified with PEG).

The 3 of the derivatives of 12-HSA (TN, DH, ME) elicited histamine release from the cells in a dose-dependant manner (n=4). However the maximum release observed varied according to the source. Thus the maximum response to TN was ca. 22% (human lung), ca. 30% (rabbit lung), ca. 38% (guinea-pig lung) and ca. 58% (rat peritoneal mast cells). DH produced ca. 50% (rat peritoneal mast cells), ca 60% (human lung) and ca 65% (guinea-pig lung). In all cell types ME produced near maximal release (60-80%). Cremophor EL was essentially without effect in all systems.

The results here presented further demonstrate the functional hetereogeneity of mast cells from different species. All the isolated cells responded to the derivatives of 12-HSA but the amount of histamine released varied between the species. In contrast, in the anaesthetized dog model neither TN nor ME produced an elevation of plasma histamine levels.

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THE EFFECT OF CUPRIC SULPHATE ON COMPOUND 48/80 AND CONCANAVALIN-A INDUCED RELEASE OF HISTAMINE FROM RAT PERITONEAL MAST CELLS.

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Earlier studies have shown that copper histidine complex has anti-inflammatory properties and at 500 µg/ml concentration has the ability to inhibit histamine release from the isolated peritoneal mast cell in the rat (West, 1981).

In the present study we have investigated if low concentrations of copper can affect Compound 48/80 and concanavalin-A induced release of histamine from isolated rat peritoneal mast cells. In addition we have studied if copper can affect spontaneous release of histamine from mast cells. Suspension of purified mast cells (approximately 10^6 cells/ml) of rats were divided into 300 µl aliquots as described earlier (Sharma & Gulati, 1985). Fifty µl of buffer was added to control aliquots and varying concentrations of cupric sulphate ($CuSO_4 5H_2O$) in 50 µl buffer were added to other aliquots. After a period of 15 minute incubation at 37°C, 50 µl buffer containing 75 ng of Compound 48/80 or 30 µg concanavalin A (plus 3µg phosphatidyl serine) was added to some of the aliquots. To the others 50 µl of buffer was added. All the tubes were then incubated for a further period of 30 minutes and histamine release in the supernatant was measured using the fluorimetric method of Shore et al (1959). The results obtained were as follows:-

$CuSO_4 5H_2O$ (mmol/ml)	Percent histamine release (mean ± SEM; n = 6)	
	Compound 48/80 induced	Concanavalin A induced
- (Unblocked)	47.68 ± 4.78	37.23 ± 2.77
4×10^{-6}	49.86 ± 6.44	40.25 ± 2.27
2×10^{-5}	26.53* ± 5.03	28.50 ± 3.00
4×10^{-5}	20.03* ± 4.64	20.23* ± 1.93

* $P < 0.05$ compared with unblocked release of histamine.

The results show that copper is a potent inhibitor of Compound 48/80 and Concanavalin-A induced release of histamine from rat peritoneal mast cells. Incubation of these cells without a releasing agent caused 9.21 ± 3.42% (mean ± SEM) release of histamine but this was not affected by the range of cupric sulphate concentrations shown in the table. Cupric Sulphate did not cause any degradation of histamine when the two were incubated at 37°C for 45 minutes. It also failed to produce any interference in the fluorimetric technique used for the estimation of histamine.

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EVIDENCE THAT IT IS THE ELEVATION OF cAMP IN A PDE III SENSITIVE POOL THAT RESULTS IN INHIBITION OF PLATELET EXCITATORY RESPONSES.

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SK&F 94120 a selective inhibitor of phosphodiesterase type III (PDE III) elevates cAMP in platelets and inhibits responses evoked by collagen, U44069 and ADP (Gristwood et al, 1986).

We have also investigated the effects of the non-selective phosphodiesterase inhibitor 3-iso butyl-1-methyl-xanthine (IBMX) on collagen-evoked aggregation and secretion and on elevation of cAMP.

IBMX had little inhibitory effect on collagen-evoked aggregation and secretion but was as effective as SK&F 94120 in elevating cAMP. If SK&F 94120 is acting soley as a selective PDE III inhibitor these results suggest that it is the elevation of cAMP regulated by PDE III that results in inhibition of agonist evoked responses.

Aggregation and secretion were measured in either a HCl aggregometer or a Coulter Lumi-aggrometer. cAMP was measured by RIA (N.E.N.) in TCA extracts from human platelets resuspended in physiological saline. SK&F 94120 inhibited aggregation and ATP secretion evoked by 5 μg/ml collagen with IC_{50}'s of 37 ± 9 (s.e.m.) and 34 ± 6 μM. IBMX at 1 and 10 μM had little inhibitory effect on collagen-evoked aggregation or secretion but at 100 μM reduced collagen-evoked aggregation and secretion to 85 ± 8 and 76.7 ± 4.3 % of control respectively (n=8). SK&F 94120 elevates cAMP in a concentration dependent manner, 100 μM, giving 87.1 ± 15 % increase in cAMP over resting. IBMX also elevated cAMP in a concentration dependent manner; giving a 92 ± 17.2 % increase in cAMP at 100 μM (n=6).

These results could be explained by IBMX elevating cAMP in a sub-population of cells where elevated cAMP has little inhibitory effect on functional responses, and SK&F 94120 elevating cAMP in another population of cells where elevated cAMP does prove to be inhibitory. Alternatively IBMX could inhibit the transduction of elevated cAMP into inhibition of functional responses, such as would occur if IBMX inhibited a cAMP dependent protein kinase.

However, we believe the most probable explanation of these results is that cAMP is compartmentalised in the platelet, in some way as yet obscure, as suggested for cardiac myocytes (Buxton and Brunton, 1983) and that the PDE III compartment is more relevant to excitatory responses.

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Gristwood R.W., Rink T.J. and Simpson A.W.M. (1986) Br. J. Pharmac 87 11P.

IDENTIFICATION AND SELECTIVE INHIBITION OF A NEW PHOSPHODIESTERASE ACTIVITY FROM CAT CARDIAC VENTRICLE.

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In the last decade a range of compounds which appear to selectively inhibit the various isoenzymes of phosphodiesterase (PDE) have been identified. Such compounds offer an opportunity to study the role of the PDE isoenzymes in controlling discrete pharmacological responses. In a separate report (Gristwood et al (1986) this meeting) the pharmacological effects of M&B 22,948 (Zaprinast), Rolipram (ZK 62,711) and SK&F 94120 on cat heart have been described.

To understand the pharmacological effects of these compounds it is necessary to characterize the selectivity of these inhibitors for PDE isoenzymes from cat heart.

Cat heart ventricle was found by DEAE-Sepharose chromatography to contain three major PDE isoenzymes. Peak 1 was characterised as a Ca^{2+}/calmodulin-stimulated enzyme which could hydrolyze both cAMP and cGMP. Peak II activity had properties which had not previously been described for a cardiac ventricular PDE. This activity showed a marked preference for cAMP as a substrate with a Km of 6 mM for cAMP and approx 40 mM for cGMP.

Peak III enzyme was the activity described by many workers as the 'low Km' PDE, having a Km of 0.6 mM for cAMP and Km of 2.9 mM for cGMP. Zaprinast, Rolipram and SK&F 94120 were found to be selective inhibitors of Peak I, II and III activity respectively. The K_i values for these compounds against the three cat ventricular PDE isoenzymes are shown in the table below. Previously, it was unclear whether Rolipram was an inhibitor of the same PDE activity inhibited by SK&F 94120, a compound in the class known as the 'low Km' PDE III inhibitors. In this study a Rolipram-sensitive PDE and a SK&F 94120-sensitive PDE have clearly been resolved.

The different pharmacological effects of these three inhibitors (Gristwood et al (1986), this meeting) on the cat heart can be explained by the different selectivities of Zaprinast, Rolipram and SK&F 94120 for three PDE isoenzymes identified in cat cardiac ventricle.

K_i values (mM) of compounds against cat cardiac ventricle phosphodiesterase.

	Peak 1	Peak 2	Peak 3
Zaprinast	6.1	140	150
Rolipram	>100	0.4	34
SK&F 94120	>1000	>100	4.4

Gristwood, R.W., Sampford, K.A. and Williams, T.J. (1986) Presented at this meeting.

THE EFFECT OF SK&F 94120, ROLIPRAM AND M&B 22948 ON FORCE OF CONTRACTION AND CYCLIC NUCLEOTIDE CONTENT OF CAT MYOCARDIUM.

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M&B 22948 (M&B), Rolipram (RP) and SK&F 94120 (SK&F) have been shown to selectively inhibit phosphodiesterase (PDE) enzymes (Peak I, II & III respectively) found in cat ventricular myocardium (England et al. 1986). The purpose of this investigation was to determine the effects of selective inhibition of each of these PDE enzyme types on force of contraction (Fc) and intracellular cyclic nucleotide content using cat isolated right ventricle strips.

Pre-weighed ventricle strips were set up under 1g tension in 50 ml organ baths containing gassed (95%O_2, 5%CO_2) Krebs solution at 37°C. The preparations were electrically stimulated (threshold voltage + 50%, 1Hz), and isometric tension was monitored. Cumulative dose-response curves (dose interval 10 min) were constructed to:- 1) M&B, 2) RP, 3) SK&F, 4) SK&F with RP at 30 μM, 5) SK&F with M&B at 30 μM, 6) M&B with RP at 30 μM. 10 mins after the final dose all tissues were freeze-clamped, immediately homogenised in 6% TCA and centrifuged. The supernatant was removed and stored frozen at -30°C. Cyclic AMP (cAMP) and cyclic GMP (cGMP) were assayed using radioimmunoassay (Du Pont).

SK&F caused a concentration-related increase in the Fc with a significant increase in cAMP content. SK&F + RP (30 μm) increased Fc significantly greater than SK&F alone (as previously reported in guinea-pig ventricle, Gristwood & Owen, 1986), and significantly increased the cAMP and cGMP content. SK&F + M&B (30 μM) caused a decrease in the SK&F Fc response and significantly increased cGMP content. M&B and RP alone and in combination had no inotropic effect, although both significantly increased cAMP and cGMP. Although elevation of the content of both cyclic nucleotides by RP + M&B (30 μM) was observed, only the increase in cGMP attained statistical significance.

Thus the elevation of cAMP resulting from the inhibition of PDE peak II in cat myocardium is not accompanied by a positive inotropic effect, whereas selective PDE III inhibition does result in an increase in Fc. Selective PDE I inhibition appears to affect primarily cGMP levels; no inotropic effect is seen. The results also indicate that the positive inotropic effect of PDE III inhibition can be significantly modified by simultaneous inhibition of either PDE I or PDE peak II enzymes.

Table 1

Drug Treatment	% Change in Fc	n	cAMP fmoles/mg wet wt Control	cAMP fmoles/mg wet wt Treated	n	p	cGMP fmoles/mg wet wt Control	cGMP fmoles/mg wet wt Treated	n	p
SK&F(100μM)	113±10.4	7	348±42	467±61	7	**	4.8±1.4	5.3±1.4	6	NS
RP+SK&F(100μM)	173.8±13.6	6	463±38	786±65	6	*	5.5±0.3	11.9±1.9	6	*
M&B+SK&F(100μM)	77±11.2	11	516±14	614±44	10	NS	3.8±0.5	9.6±1.5	11	**
M&B(30μM)	-3.7±4.9	11	349±28	413±41	11	*	3.9±0.4	8.7±1.0	8	**
RP(30μM)	1±6.5	10	383±22	476±30	9	**	5.4±0.4	8.9±1.3	8	*
RP+M&B(30μM)	-7.9±3.8	10	516±16	610±54	8	NS	3.8±0.6	9.6±1.8	10	**

NS = Not significant, * = $p<0.05$, ** = $p<0.01$

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