EVIDENCE FOR A PRESYNAPTIC A_1 ADENOSINE RECEPTOR IN THE RABBIT PORTAL VEIN

C.M. Brown, M.G.Collis, & K. Titley, Imperial Chemical Industries PLC, Pharmaceutical Division, Alderley Park, Macclesfield, Cheshire SK10 4LF.

Adenosine and the adenine nucleotides can modify neurotransmission at a number of synapses (Hedquist & Fredholm, 1976; Vizi, 1979; Paton 1981) and appear to mediate their effects through a presynaptic adenosine (P1) receptor (Burnstock 1978). A sub-classification of adenosine receptors based on their effects on adenylate cyclase and on ligand binding studies has been proposed (Van Calker et al 1979; Daly et al 1981). At A1 receptors the potency of 5'-substituted and N^6-substituted analogues of adenosine is similar, but the receptor exhibits stereospecificity for N^6-phenylisopropyladenosine (PIA), L-PIA being 50-100 times more potent than its D-diastereoisomer. Activation of the A1 receptor leads to an inhibition of adenylate cyclase activity. At A2 receptors the 5'-substituted analogues of adenosine are more potent than the N^6-substituted analogues, but the difference in potency between D- and L-PIA is at the most five fold. A2 receptor activation mediates stimulation of adenylate cyclase activity. Both A1 and A2 receptors are antagonised by methylxanthines. The purpose of the present study was to determine which of these sub types of adenosine receptor is present on the adrenergic nerve terminals in the portal vein.

Longitudinal strips of rabbit portal vein were mounted in Krebs solution at 37°C for isometric recording. The tissues were stimulated with a 4 minute train of pulses (2Hz, 20 volts, 1msec) delivered through platinum strip electrodes. A comparison of the effects of the purines on matched responses evoked by 2Hz field stimulation and by exogenous noradrenaline was made in each preparation. This gave an indication of any post-synaptic component of the response. The response to field stimulation was blocked by the administration of either guanethidine (5×10^{-6}M) or phentolamine (5×10^{-6}M).

Adenosine, 2-chloroadenosine, 5'-N-ethylcarboxamide adenosine (NECA), 5'-N-cyclopropylcarboxamideadenosine (NCPCA), L-PIA, D-PIA, and N^6-cyclohexyladenosine(CHA) all elicited concentration dependent inhibitions of the response to field stimulation. The order of agonist potency assessed at the ED_{20} level was L-PIA=CHA=NCPCA=NECA>2-chloroadenosine>adenosine>D-PIA. There was no significant post synaptic effect of any of these compounds at the ED_{20} dose. The difference in potency between D- and L-PIA was approximately 100 fold.

8,phenyltheophylline is a purine receptor antagonist with little inhibitory effect on phosphodiesterase (Smellie et al 1979). This methylxanthine (10 μM) significantly attenuated the responses to adenosine and all its substituted analogues.

These findings indicate that adenosine and its 2-,5' and N^6-substituted analogues are potent presynaptic inhibitors of noradrenaline release. The relative potencies of the adenosine analogues, and the stereospecificity of the receptor to D- and L-PIA suggests that the adenosine receptor belongs to the A1 sub type.

Burnstock,G.(1978) "Cell membrane receptors for drugs and hormones" ed Bolis,L. & Straub, R.W. pp 107-118. New York. Raven Press.
Daly,J.W., Bruns, R.F. & Snyder, S.H. (1981). Life Sci.28. 2083-2097.
Hedquist,P. & Fredholm, B.B.(1976) Naunyn-Schmiedebergs Arch.Pharmacol.293, 217-223.
Paton, D.M. (1981) "Purinergic Receptors" ed Burnstock, G. pp 199-219. London. Chapman & Hall.
Van Calker, D., Muller, M. & Hamprecht, B. (1979) J.Neurochem. 33, 999-1005.
Vizi, E.S. (1979). Prog.Neur biol. 12, 181-290.

THE EFFECTS OF α_1 AND α_2 AGONISTS AND ANTAGONISTS ON RAT SPLENIC SMOOTH MUSCLE STRIPS

M.I. Ahmed & I.L. Naylor (Introduced by G.D.H. Leach) Postgraduate School of Pharmacology, University of Bradford, Bradford, BD7 1DP.

Isolated spleen strips from rabbits, cats and guinea pigs have been used in vitro to study pre and post-synaptic receptors (Celuch, Dubocovich and Langer, 1978), adrenergic blocking agents (Bickerton, 1963) and agonist/antagonist potencies (Digges, McPherson and Summers, 1981). In contrast studies using the laboratory rat are seldom described (Harper and Hughes, 1978) and this study was undertaken to indicate the suitability of using rat spleen for such investigations on the basis of three determinations:- (a) the optimal experimental conditions for rat spleen strips, (b) the responsiveness to electrical stimulation and (c) the effects of a range of agonists/antagonists upon the preparation.

Spleens from two groups of male, CFE (Bradford Strain) rats (a) 250 to 380 g (b) 500 to 700 g and one female group, 300 to 380 g were removed after stunning and exsanguination. Spleens were then either (a) longitudinally bisected or (b) cut transversely into 3 mm wide strips from the central region of the spleen and then suspended on a tissue holder with parallel platinum electrodes in one of six physiological solutions (Bretag, De Jalon, Krebs-Hensleit, McEwen, Ringer Locke and Tyrode solution) attached to either an isometric strain gauge (Pye Dynamics TS200) under a resting tension of 1 g or an auxotonic transducer (Illingworth and Naylor, 1982) (load $\equiv$ 1 g displacement) using stainless steel wire (37 swg) in an overflow bath system and equilibrated for 1 hr.

In a series of experiments to choose the most suitable physiological solution Bretag was decided upon since the response to electrical stimulation was maintained for a period of at least 4 hrs and sensitivity to adrenaline (AD), noradrenaline (NA) and phenylephrine (PE) was also maintained.

Isolated spleen strips from all three groups were found to be insensitive to Acetylcholine (ACh) 1×10^{-7} to 5×10^{-5}M. Whereas NA, AD and PE all produced contractile responses with the rank order of potency being AD>NA>PE. Electrical field stimulation (1 m sec pulse width, supramaximal voltage, 1-25Hz) produced frequency dependent and maintained contractile responses in all experimental groups except for the female animals. The response to field stimulation were competitively antagonised with phentolamine (5×10^{-7}M) and abolished with guanethidine (1×10^{-5}M) or reserpine (1 mg Kg^{-1} s.c. 18 hrs prior to use) pretreatment. Field stimulation in the presence of oxytetracycline (1×10^{-4}M) ascorbic acid ($2\text{-}8 \times 10^{-4}$M) EDTA ($4 \times 10^{-5}$M) corticosterone ($8.6 \times 10^{-5}$M) did not significantly alter the effect of either NA addition ($P>0.05$) or field stimulation ($P>0.05$). In the presence of clonidine (1×10^{-8} - 1×10^{-7}M) and guanabenz (1×10^{-9} - 1×10^{-6}M) the response to stimulation in the range 1-6Hz was significantly ($P<0.05$) decreased whereas yohimbine (5×10^{-7}M) failed to produce a significant alteration ($P>0.05$).

It is considered that rat splenic smooth muscle contain adrenergic receptors sensitive to both clonidine and guanabenz but not yohimbine an effect which is being investigated further.

Bickerton, R.K. (1963). J. Pharmacol. Exp. Ther. 142, 99-110.
Celuch, S.M., Dubocovich, M.L. & Langer, S.Z. (1978) Br. J. Pharmac. 63, 97-109.
Digges, K.G., McPherson, G.A. & Summers, R.J. (1981) J. Auton. Pharmac.1,313-320.
Harper, B., Hughes, I.E. & Noormohamed, F.N. (1978) J. Pharm. Pharmac. 30,167-172.
Illingworth, D.R. & Naylor, I.L. (1982) J. Pharmacol. Meth. (in press)

EFFECTS OF METOCLOPRAMIDE AND DOMPERIDONE ON CHOLINERGIC RESPONSES IN RAT ISOLATED STOMACH

Christine M. McClelland and G.J. Sanger, Beecham Pharmaceuticals, Medicinal Research Centre, Coldharbour Road, The Pinnacles, Harlow, Essex. CM19 5AD

Metoclopramide (Mcp) could increase gastric motility by potentiation of cholinergic-induced contractions, acting by a mechanism which may be independent of adrenoceptors (Anderson et al, 1977). However an interaction of Mcp with adrenoceptors is suggested by its antagonism of the inhibition by clonidine of contractions to cholinergic nerve stimulation (McClelland and Sanger, 1982). Increased gastric motility with domperidone might, at least partly be due to antagonism of α_1-adrenoceptor or dopamine-induced responses (Sahyoun et al, 1982). We have now looked for effects of domperidone on gastric cholinergic contractions and compared the results with metoclopramide.

Strips of rat gastric fundus were cut parallel to the longitudinal muscle (Bennett et al, 1980). Contractions to electrical field stimulation (EFS; Crema et al, 1968; bipolar 0.5ms rectangular pulses; 5Hz rate; 20s train; maximum current 1A/electrode [80-120V/cm]; 10 min cycle) could be blocked with tetrodotoxin 0.1μM or atropine 1.4μM, indicating predominant cholinergic activation. Mcp 2.8-282μM slightly increased muscle tone in 3 of 8 preparations. Contractions to EFS were increased with 0.03-28μM Mcp but reduced with 282μM Mcp. Domperidone 0.003-2.8μM had no effect on muscle tone or on contractions to EFS, but higher concentrations inhibited the contractions (Table 1). Vehicle controls had no effect.

Table 1: % Increase (medians with semiquartile ranges in parenthesis) in contractions to EFS. *P<0.05, **P<0.01, Wilcoxon matched pairs comparison with control. n = number of preparations.

Drug (μM)	Metoclopramide (n = 8)	Domperidone (n = 6)
0.003	12 (4 to 15)	4 (-11 to 13)
0.03	19 (3 to 24)*	0 (-8 to 9)
0.3	43 (16 to 70)*	12 (-2 to 21)
2.8	99 (59 to 139)**	6 (-2 to 21)
28.0	169 (89 to 320)**	-54 (-100 to -16)**
282.0	-70 (-81 to -21)*	-100 **

Submaximal contractions to ACh (30s contact, 10 min cycle; contractions approx 50% maximum) were unaffected with 0.003-28μM Mcp (P>0.05) and reduced with 282μM Mcp (P<0.05; n = 8 for each). Similar results were obtained using 8-24% maximum contractions to ACh (n = 3).

Domperidone had no excitatory action on cholinergic responses, as reported by Lefebvre and Willems (1981), but just with 10μM domperidone. The results with Mcp confirm those of Anderson et al (1977), but also show that Mcp may be active in the low concentrations detected in blood plasma after a therapeutic dose (Bateman et al, 1978). Mcp did not increase contractions to ACh or reduce the relaxations to non-adrenergic inhibitory neurones revealed with EFS in the presence of atropine (Anderson et al, 1977; and unpublished). Metoclopramide may therefore increase ACh release in rat stomach.

Anderson, J.D. et al (1977) J. Pharm. Pharmac. 29, 53P
Bateman, D.N. et al (1978) Br. J. clin. Pharmac. 6, 401-407
Bennett, A. et al (1980) Br. J. Pharmac. 71, 169-175
Crema, A. et al (1968) Gut, 9, 633-637
Lefebvre, R.A. & Willems, J.L. (1981) Br. J. Pharmac. 72, 537P
McClelland, C.M. & Sanger, G.J. (1982) This meeting
Sahyoun, H.A. et al (1982) J. Pharm. Pharmac. 34, 27-33

THE EFFECTS OF COCAINE, DESMETHYLIMIPRAMINE, AND 6-HYDROXYDOPAMINE ON DRUG-INDUCED CONTRACTIONS OF MOUSE ANOCOCCYGEUS

A. Gibson & O. Yu, Department of Pharmacology, Chelsea College, Manresa Road, London SW3 6LX.

In order to obtain reliable quantitative estimates of drug-adrenoceptor interaction it is necessary to inhibit the neuronal amine uptake system (Furchgott, 1972 ; Kenakin & Beek, 1981). In this study we have investigated the use, in vitro, of cocaine, desmethylimipramine (DMI), and 6-hydroxydopamine (6OHDA) to reduce the influence of neuronal uptake on postsynaptic α-adrenoceptor responses of the mouse anococcygeus muscle.

Male mice (LACA ; 22 - 33g) were stunned and bled. The anococcygeus muscles were dissected and set up in 1 ml organ baths as described previously (Gibson & Wedmore, 1981). Cocaine and DMI were included in the Krebs bathing medium and were in contact with the tissue for 30 min before testing agonist potency. 6OHDA (800 μM) was incubated with the tissue for 2 h, after which the tissue was washed with drug-free Krebs solution every 5 min for 30 min before testing agonist potency. Isometric contractile responses were obtained to the α-adrenoceptor agonists noradrenaline (NA) and methoxamine, and to the muscarinic receptor agonist methacholine. Horizontal shifts of agonist dose-response curves, compared with controls, were measured at the EC_{50} level.

Cocaine (0.2, 0.5, 2, and 5 μM) produced a dose-dependent leftward displacement of the NA dose-response curve (2, 6, 13, and 16 fold shift respectively) with no change in maximum response. However, at concentrations above 2 μM, cocaine itself produced muscle contraction. This was due, at least in part, to release of NA from sympathetic terminals since the effect was reduced in tissues taken from mice pretreated with reserpine (5 mg per kg ; ip ; 24 h). 2 μM cocaine had no effect on contractile responses to methoxamine or methacholine.

DMI (5, 10, 30, and 50 nM) also produced dose-dependent leftward displacements of the NA dose-response curve (4, 8, 16, and 21 fold shift respectively) with no change in maximum response. However, at 100 nM DMI this trend was reversed, the leftward shift being reduced to 11 fold. In addition, 100 nM DMI caused a rightward displacement of the dose-response curves to methoxamine and methacholine (3 fold shift in both cases with no change in maximum response). The pA_2 of DMI measured against methoxamine was 7.4 (slope of Schild plot - 0.76).

Following incubation with 6OHDA the slopes of the dose-response curves and the maximum responses of the tissue to both NA and methacholine were reduced.

In conclusion, each of the methods used in this study to reduce the influence of neuronal uptake on drug-induced contractions of the mouse anococcygeus was associated with problems. The optimal concentration of cocaine, which produced a selective increase in sensitivity to NA, was 2 μM ; above this release of NA caused contraction. The use of DMI was complicated by concurrent uptake blockade and postsynaptic depression (Kenakin & Beek, 1981). Incubation of the tissue with 6OHDA proved unsatisfactory due to non-selective postsynaptic depression.

Furchgott, R.F. (1972) Handbook of Experimental Pharmacology. 33, 283-335.
Gibson, A. & Wedmore, C.V. (1981) J. Auton. Pharmac. 1, 225-233.
Kenakin, T.P. & Beek, D. (1981) J. Pharmac. exp. Ther. 219, 112-120.

EFFECTS OF AZEPEXOLE ON THE RAT ISOLATED ANOCOCCYGEUS

Jill Coates & D.F. Weetman, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Sunderland Polytechnic, Sunderland, Tyne and Wear, SR1 3SD.

Azepexole has been shown to be a selective agonist for α_2-adrenoceptors located at both pre and post-junctional sites in the pithed rat (Pichler et al, 1980; Timmermans and Van Zwieten, 1980). Azepexole has now been examined on the rat anococcygeus in vitro.

Preparations were dissected from male Sprague-Dawley rats (270-320g), and were suspended in McEwen's solution (gassed with 95% O_2: 5% CO_2) at 37 ± 1°C. Tension in the anococcygeus was measured isometrically and was initially set at 0.5g. Contractions were evoked on some preparations by field stimulation (10Hz for 0.7s, 500μs pulses at 140V. Agonist drugs were applied cumulatively, only one concentration-response curve being obtained from each muscle. Experiments were performed on pairs of preparations, one serving as a control for the other that received an antagonist.

Field stimulation of the anococcygeus produced a monophasic twitch response (2.86 ± 0.13g, n = 15). Azepexole (5.5-550nM) inhibited the twitch response (up to 100%), which could be reversed (80-90%) by yohimbine (30-140nM). Within this concentration range, yohimbine did not alter twitch height, but did antagonise the inhibitory effect of azepexole (pA_2 for yohimbine = 7.86 ± 0.22, with a slope 1.3 not significantly different from 1, P>0.05, n =12). High concentrations of yohimbine (>150nM) reduced the response of the anococcygeus to field stimulation. The comparitively low concentrations of azepexole that were employed for the pA_2 determinations neither contracted the muscle nor reduced the sensitivity of exogenous noradrenaline (NA control maximum = 6.45 ± 0.6g, EC50 412 ± 59nM: in the presence of azepexole maximum = 6.0 ± 0.5g, P>0.05, EC50 = 443 ± 30nM, P>0.05, n = 5). High concentrations of azepexole (>10μM) contracted the anococcygeus (maximum = 1.24 ± 0.37g, n = 7), and concurrently reduced the maximum response to NA by 49%, and the sensitivity 104 fold. This postjunctional partial agonist effect of azepexole (intrinsic activity relative to NA = 0.19) was absent in preparations pretreated with phenoxybenzamine (0.3nM for 30min followed by 20 washes over 30min) or in the presence of prazosin (10nM).

Unlike Sgd 101/75 (Coates et al, 1982), azepexole reduced the maximum effect of the anococcygeus to NA in phenoxybenzamine pretreated preparations (from 4.66 ± 0.57g to 3.38 ± 0.5g, n = 5, P>0.05). Therefore azepexole does not contract the anococcygeus by an action on α_{1S} -adrenoceptors, but does interact with α_1-adrenoceptors. However, the predominant action of azepexole is to stimulate prejunctional α_2-adrenoceptors.

Acknowledgements. We are grateful to Boehringer Ingelheim for the azepexole and Pfizer for the prazosin used in these experiments.

Coates,J. et al (1982). Br.J.Pharmac., 75, 549-552.
Pichler, L. et al (1980). Eur. J.Pharmac., 65, 233-241.
Timmermans, P.B.M.W.M. and van Zwieten, P.A. (1980). Arch.Pharmac., 313, 17-20.

A SPASMOLYTIC ACTION OF BILE SALTS ON THE GUINEA-PIG GALL BLADDER IN VITRO

Harper, A.A.[1], Hood, A.J.C., Mushens, J., Smy, J.R. and Veitch, R.K.[1] (Introduced by D.F. Weetman). Department of Pharmacology, Sunderland Polytechnic, Sunderland SR1 3SD and [1]Department of Pharmacological Sciences, The University, Newcastle upon Tyne, NE1 7RU.

Pancreotone, a polypeptide in crude extracts of ileal and colonic mucosae, inhibits exocrine secretion of the pancreas stimulated by secretin and CCK-PZ (pancreozymin, Boots) and contractions of the gall bladder stimuated by CCK-PZ in anaesthetised animals (Harper et al, 1979). In urethane-anaesthetised guinea-pigs, in which contractions of the gall bladder were obtained to separate single injections of CCK-PZ, pancreotone (1.0 unit i.v.) produced a parallel shift to the right in the dose-response curve to CCK-PZ (range 0.25 - 1.0 units i.v.). The percentage inhibition of contractions in response to injections of constant doses of CCK-PZ was also linearly related to the log dose of pancreotone injected (0.25 - 2.0 units i.v.).

In an attempt to produce a rapid assay, the inhibitory action of pancreotone was studied using the in vitro guinea-pig gall bladder suspended in Ringer Tyrode (at 30°C, gassed 95% O_2 : 5% CO_2). Although crude extracts of pancreotone added before or during contractions (recorded via a Grass FT10 force displacement transducer) induced by CCK-PZ, caerulein, histamine, choline esters and Ba^{++} inhibited the contractile responses, inhibitory actions were found also with extracts made from upper regions of the gut which were not inhibitory in vivo. During sustained, submaximal contractions to acetylcholine (33 µM), the percentage inhibition of the contraction by pancreotone (1.0 unit/ml) was (i) 54% ± 8%, mean ± s.e.mean, and using an equal weight of gastric antral extract 57% ± 8%, n = 6; (ii) 56% ± 5% and using stomach muscle extract 63% ± 7%, n = 7. Since the extracts were prepared by bile salt precipitation (approximately 30% of extract weight), comparison was made between pancreotone and the ox-pig bile salt mixtures used; pancreotone produced inhibition of 62% ± 4% and the bile salt mixture 52% ± 14% and 71% ± 12%, n = 4, assuming respectively 25% and 50% of the weight of pancreotone extract was bile salt. Cholic acid (250 µM) added during sustained responses to carbachol (33 µM) inhibited contraction by 40% ± 3%, n = 4. Similar reductions were obtained using deoxycholic, glycocholic and taurocholic acids, but not with methyl cholate (250 and 500 µM), methyl deoxycholate (0.5 and 1mM), Tween 80 (100 µg/ml) or sodium lauryl sulphate (33 µM). Pancreotone extracts freed from bile salt retain their ability to inhibit the gall bladder in vitro.

The activity of pancreotone in vivo is not due to bile salt content since (i) bile salt free extract is inhibitory (ii) bile salt is without inhibitory activity in vivo, (iii) the active component is susceptible to tryptic digestion. Pancreotone is also inhibitory against vagally stimulated gall bladder contraction and against CCK-PZ in the presence of atropine,(Harper et al, unpublished).

In conclusion, these preliminary studies show that (i) the use of in vitro preparations of the gall bladder as an assay for crude pancreotone would necessitate prior removal of bile salts, (ii) the mechanism of the spasmolytic action of bile salts in vitro may be of interest for further investigation.

Harper, A.A. et al (1979). J. Physiol. Lond. 292, 435-467.

α_2-ADRENOCEPTOR BLOCKADE PRODUCED BY SGD 101/75 IN THE RAT VAS DEFERENS IN VITRO

U.Jahn[1], N. Turner & D.F. Weetman, Department of Pharmacology, Faculty of Pharmaceutical Sciences, Sunderland Polytechnic, Sunderland SR1 3SD, Tyne and Wear and Department of Pharmacology[1], Siegfried AG, CH-4800 Zofingen, Switzerland.

Sgd 101/75 is a partial agonist for α_1-adrenoceptors (e.g. guinea-pig taenia) or a full agonist on α_{1S}-adrenoceptors (rat anococcygeus (Ismail et al 1981; Coates et al 1982). Preliminary results on the guinea-pig ileum indicated that Sgd 101/75 antagonised the inhibitory effect of noradrenaline (NA) on the twitch response to single pulse electrical stimulation (Ismail et al 1981). Since several receptors are activated by NA in producing this effect (Wood, 1982), the interaction of Sgd 101/75 on the prejunctional α_2-adrenoceptors of the rat vas deferens was investigated.

Whole vasa were dissected from Sprague-Dawley rats (250-400g) and suspended in isolated organ baths containing McEwen's solution at 37 ± 1°C (gas 95% O_2 : 5% CO_2). Contractions were induced by field stimulation (0.1Hz, 0.5ms, 140v). Experiments were performed on paired preparations, one (control) receiving clonidine (0.6 - 6000nM cumulatively at 3min intervals) and the other being treated similarly 15 min after applying Sgd 101/75 (4-400μM) or yohimbine (21-510nM). Values are the mean ± s.e.mean of n determinations.

Sgd 101/75 but not yohimbine increased the twitch height (initially 1.69 ± 0.08g, n = 31) of the vas deferens. Both Sgd 101/75 and yohimbine antagonised the inhibition of twitch height due to clonidine (Sgd 101/75 pA_2 = 6.12 ± 0.8, slope 0.95, not significantly different from 1, $P>0.05$, n = 14: yohimbine pA_2 = 8.14 ± 0.11, slope 0.92, $P>0.05$, n = 12). A very high concentration of Sgd 101/75 (400μM) did not antagonise adenosine-induced inhibition of the twitch response (DR = 2.3 ± 0.14, n = 6).

The elevation of twitch height by Sgd 101/75 was not due to α_2-adrenoceptor blockade because a) the concentration-response curve for this effect was quite different from that for the α_2-adrenoceptor block and b) yohimbine did not exert such an effect.

It is concluded that Sgd 101/75 exerts a specific but weak prejunctional competitive antagonism of α_2-adrenoceptors. This effect is additional to the partial agonist effect on α_1-adrenoceptors, and the full agonist effect on α_{1S}-adrenoceptors.

Coates, J. et al (1982). Br.J.Pharmac., 75, 549-552.
Ismail, S. et al (1981). Br.J.Pharmac., 72, 535-536P.
Wood, D. (1982). Br.J.Pharmac., Proc.Suppl. in the press.

EFFECTS OF NIFEDIPINE, VERAPAMIL AND NITROPRUSSIDE ON (^{45}Ca) EFFLUX FROM THE RAT VAS DEFERENS

D.W.P. Hay and R.M. Wadsworth, Department of Physiology & Pharmacology, University of Strathclyde, Glasgow G1 1XW.

We have previously reported that calcium channel inhibitors block ^{45}Ca uptake by the rat vas deferens through both voltage dependent channels (in low concentrations) and Ba^{2+} activated channels (in high concentrations). Methoxamine contractions in the rat vas deferens are not associated with an increase in ^{45}Ca uptake. This communication reports the effects of these drugs on ^{45}Ca efflux from the same tissue.

Intact or bisected vasa deferentia were loaded with ^{45}Ca 2μCi ml^{-1} in Krebs-Henseleit solution at 37°C for 3h, then superfused at 2.7 ml min^{-1} with Krebs-Henseleit solution containing 2.5 or 0 mM $CaCl_2$ or EGTA 0.05 mM. After 90 min the superfusate was changed to an identical solution containing the agonist. The superfusate was collected every 2 min and counted by liquid scintillation. Residual ^{45}Ca was extracted and counted in the same way.

Basal ^{45}Ca efflux occurred more rapidly into Ca-free solution than into 2.5mM Ca solution. Basal efflux was not significantly affected by nifedipine (0.29-14.4 μM), verapamil (2.04-61.1μM)or by nitroprusside (1678μM). KCl 160mM increased ^{45}Ca efflux: the initial release of ^{45}Ca was larger in the Ca-free solution than in the 2.5mM Ca solution, but the later phase of ^{45}Ca efflux was the same in both solutions. It is possible that part of the ^{45}Ca released by KCl is released from superficial high affinity sites that are displaced by extracellular Ca (Karaki & Weiss, 1980). The remainder may originate from within the cells, being transported by a mechanism distinct from the Ca channels mediating contraction (Godfraind, 1978), since KCl-stimulated ^{45}Ca efflux was not inhibited by verapamil (2.04μM) nifedipine (0.29μM) or nitroprusside (1678μM). In contrast to these results KCl-stimulated ^{45}Ca efflux from the rabbit ear artery was inhibited by methoxyverapamil 10^{-5}M (Casteels & Droogmans, 1981).

Basal ^{45}Ca efflux (into 2.5mM Ca solution) was slower from the prostatic half than from the epididymal half and the slow component of efflux was greater. This may reflect the presence of a high affinity extracellular store of Ca in the prostatic half that supports the KCl contraction in Ca-free solution (Hay & Wadsworth, 1982). KCl stimulated ^{45}Ca displaced by KCl does not come from the prostatic high affinity store.

Methoxamine 8.1μM and, to a lesser extent, $BaCl_2$ 1mM each produced a small transient increase in ^{45}Ca efflux. This may represent displacement of specific stores of Ca associated with the early part of these responses (the phasic part of the Ba^{2+} contraction probably depends on intracellular Ca; the fast part of the prostatic response to α-adrenoceptor agonists probably depends on a high affinity extra-cellular store). Ba^{2+} or methoxamine stimulated efflux was slightly increased by verapamil or nifedipine, possibly due to inhibition of ^{45}Ca backflux.

Casteels, R. & Droogmans, G. (1981) J. Physiol. 317, 263-279
Godfraind, T. (1978) In Progress in Pharmacology Vol. 2 pp 57-68, van Zwieten P.A. & Schonbaum, Z. Eds., Gustav Fischer Verlag, Stuttgart
Hay, D.W.P. & Wadsworth, R.M. (1982) Br. J. Pharmac. 76, 103-113
Karaki, H. & Weiss, G.B. (1980) J. Pharmac. Exp. Ther. 215, 363-368

PHARMACOLOGICAL CHARACTERISATION OF ASCARIS ALLERGEN INDUCED BRONCHOCONSTRICTION IN DOGS

Jackson, D.M., Richards, I.M. and Vendy K, Pharmacology Department, Fisons plc, Pharmaceutical Division, Loughborough, Leicestershire, LE11 0QY.

One in vivo immunological experimental model of asthma is the airway response to inhalation of Ascaris suum antigen in 'naturally' sensitive dogs, (Booth et al, 1970). Sensitivity probably occurs as a result of prior infestation with cross reacting, antigenically related nematodes. Serum from these dogs could be used to passively transfer skin reactivity to Ascaris and the serum antibodies responsible were heat labile and non precipitating suggesting that they may belong to the IgE class (Booth et al, 1970).

In this study we have investigated the actions of salbutamol, atropine and sodium cromoglycate on Ascaris induced bronchoconstriction in an attempt to characterise the nature of the response. Inhalation of Ascaris at weekly intervals produced reproducible increases in total lung resistance (RL) and falls in dynamic lung compliance (Cdyn) in 10 anaesthetised (chloralose) artifically ventilated dogs. Changes in RL and Cdyn usually reached a maximum 4-6 minutes after allergen inhalation and returned to control values after 30 minutes.

Inhalation of salbutamol produced a dose-related inhibition of Ascaris-induced bronchoconstrictions in two dogs (10 breaths generated from a 1.0% w/v solution abolished Ascaris induced changes in RL and Cdyn). Efferent vagal blockade with atropine, 0.25 mg/kg i.v. during an established bronchoconstriction reversed 4.5 + 4.2% of the induced RL change and 9.4 +2.8% of the induced Cdyn change. The antihistamine, chlorpheniramine (maximum dose 1.0 mg/kg) completely abolished histamine-induced changes in RL and Cdyn but did not affect the response to Ascaris. Sodium cromoglycate given by aerosol (0.001 - 4.0% w/v) or i.v. did not affect the Ascaris induced changes in lung mechanics.

The effectiveness of salbutamol suggests that contraction of airway smooth muscle rather than mucosal oedema or excess mucus production is responsible for the changes in RL and Cdyn which occur. The mechanism by which Ascaris induces this contraction is controversial. Some workers (Gold et al, 1972) claim that a vagally mediated reflex, cholinergic component is responsible whilst others (Krell et al, 1976) claim that bronchoconstriction occurs as a result of direct contraction of airway smooth muscle. The effectiveness of atropine on RL changes suggests that reflex mechanisms are involved in the central airway response to Ascaris, and the minor effect on Cdyn changes suggests that Ascaris-induced changes in Cdyn are due to the direct contraction of peripheral airway smooth muscle by the mediators of anaphylaxis. The lack of a reflex component in the experiments of Krell et al (1976) may be explained by the use of pentobarbitone as an anaesthetic.

The role of histamine as a mediator of Ascaris-induced bronchoconstriction is equivocal. The lack of effect of antihistamines in these experiments is inconsistent with the findings that histamine can be detected in vivo in the arterial blood of dogs after inhalation of Ascaris antigen (Chiesa et al, 1975).

The lack of inhibition with sodium cromoglycate is also difficult to explain. Sodium cromoglycate has been shown to inhibit reaginic antibody mediated reactions in a number of species. The possibility that the Ascaris-induced bronchoconstriction in 'naturally' sensitive dogs is not mediated by a reaginic antibody/mast cell mechanism must be considered.

Booth, B.H. et al (1970). J Lab. Clin Med. 76, 181-189.
Chiesa, A. et al (1975). Am. Rev. Resp. Dis. 111, 148-156.
Gold, W.M. et al (1972). J. app Phys. 33, 719-725.
Krell, et al (1976). All. Clin. Immunol. 58, 19-30.

DIFFERENCES BETWEEN THE THROMBOXANE A_2 RECEPTORS ON RABBIT AND HUMAN PLATELETS

Lorraine Anderson and D.E. MacIntyre, Department of Pharmacology, University of Glasgow, Glasgow G12 8QQ, Scotland.

Prostaglandin (PG) G_2, PGH_2 and thromboxane (Tx) A_2 are potent inducers of human platelet activation. That these effects are mediated by combination with specific receptor(s) in/on platelets is indicated by a comparison of the structure-activity relationships of agonists, by the use of selective antagonists, by homologous desensitization studies and by radioligand binding analyses (MacIntyre, 1981; Armstrong et al., 1982). Certain compounds act as TxA_2 agonists on aortic tissue but act as TxA_2 antagonists on platelets, indicating that TxA_2 receptors are different in platelets and in vascular smooth muscle (Lefer et al., 1980). To investigate the possibility that there might be differences between the TxA_2 receptors on platelets of different species, we compared the structure-activity relationships of prostanoid (TxA_2-like) agonists and the effects of putative TxA_2 antagonists on rabbit and human platelets.

Platelet aggregation was measured photometrically in 0.17ml samples of human or rabbit citrated platelet-rich plasma (PRP). Agonists used were:- 15(S)-hydroxy-11α,9α-epoxymethano-prostadienoic acid (U46619); 11-deoxy-PGE_2 (Wy 18189); 11-deoxy-11-α-methyl-PGE_2 (Wy 16868); 11-deoxy-15(RS)-15-methyl-PGE_2 (Wy 17186) and 11-deoxy-15(S)-15-methyl-PGE_2 (Wy 40659). The absolute potency and the rank order of potency of agonists (U46619 > Wy 40659 > Wy 18189 > Wy 17186 > Wy 16868) was the same in PRP of both species. We then examined the effects of several compounds known to act as antagonists at human platelet TxA_2 receptors (MacIntyre, 1981; Jones et al., 1982). Mean I_{50} values (n=3) for inhibition of U46619-induced human platelet aggregation were:- Trimethoquinol (3μM); 13-Azaprostanoic acid (3μM); EP 045 (10μM); Azo Analogue I (6μM) and 9,11-Epoxyimino-prostadienoic acid (0.3μM). In contrast, the mean I_{50} values (± S.E., n=4) for inhibition of rabbit platelet aggregation induced by U46619 and Wy 40659 respectively were:- Trimethoquinol (> 300μM; > 300μM); 13-Azaprostanoic acid (> 180μM; > 180μM); EP 045 (44±9μM; 50±3μM); Azo Analogue I (55±1μM; 48±4μM) and 9,11-Epoxyimino-prostadienoic acid (50±6μM; 44±11μM).

These results indicate that rabbit and human platelets respond similarly to a range of TxA_2 mimetics, but differ markedly in their sensitivity to putative TxA_2 antagonists. Possible inter-species variations in platelet TxA_2 receptors should be considered when evaluating TxA_2 antagonists in animal models of thrombosis.

This study was supported by the Medical Research Funds of the University of Glasgow.

Armstrong, R.A. et al. (1982) Proceedings of the Fifth Int. Congress on Prostaglandins, p. 672
Jones, R.L. et al. (1982) Br.J.Pharmac., in press
Lefer, A.M. et al. (1980) Proc.Natl.Acad.Sci. U.S.A. 77, 1706-1710
MacIntyre, D.E. (1981) In Platelets in Biology and Pathology 2. (ed. J.L. Gordon), 211-247

ENHANCED PRODUCTION OF PROSTACYCLIN IN BLOOD AFTER TREATMENT WITH SELECTIVE THROMBOXANE SYNTHETASE INHIBITOR, UK-38,485

M.J. Parry, M.J. Randall, E. Hawkeswood, P.E. Cross and R.P. Dickinson
Pfizer Central Research, Sandwich, Kent, CT13 9NJ.

A balance between the opposing biological effects of thromboxane A_2 (TxA_2) and prostacyclin (PGI_2) may be important in normal vascular homeostasis. In some cardiovascular diseases associated with thromboembolism and vasospasm the generation of TxA_2 is increased, while that of PGI_2 is decreased. It has been proposed that an agent which can re-direct the TxA_2/PGI_2 balance towards normal may have therapeutic value (Moncada and Vane, 1978).

An example of such an agent is a selective inhibitor of TxA_2 synthetase and we have recently described the effects of a compound of this type, dazoxiben hydrochloride (UK-37,248-01) in animals (Randall et al, 1981) and in man (Tyler et al, 1981). We now report the identification of another imidazole derivative, UK-38,485, 3-(1H-Imidazol-1-yl methyl)-2-methyl-1H-indole-1-propanoic acid. This compound retains the selectivity of action of dazoxiben but has the advantages of greater potency and a longer duration of action.

UK-38,485 is a potent inhibitor of human blood platelet microsomal TxA_2 synthetase, $IC_{50} = 1.8 \times 10^{-8}M$. In contrast, concentrations up to $1 \times 10^{-4}M$ have negligible effects on PG endoperoxide synthesis by ram seminal vesicle microsomes and on PGI_2 synthesis by pig aortic microsomes.

The effect of UK-38,485 on arachidonate metabolism in whole animals was studied by determining levels of TxB_2 and 6ketoPGF$_1\alpha$ generated *in vitro*, during clotting of blood samples taken from animals which had received the compound. Serum TxB_2 and 6ketoPGF$_1\alpha$ were determined using specific radioimmunoassays (Randall et al, 1981). In anaesthetised rabbits, 45 minutes after injection of UK-38,485 at 1mg/kg i.v., serum TxB_2 levels were reduced by 88%, whereas there was a 4.0-fold rise in 6ketoPGF$_1\alpha$. In conscious dogs serum TxB_2 was reduced by almost 90% for 6 hours following an oral dose of 1mg/kg with 50% inhibition still apparent after 15 hours. This profile of reduced TxB_2 and elevated 6ketoPGF$_1\alpha$ in the serum samples was obtained consistently during a ten day study in dogs receiving a single daily dose of UK-38,485 at 1mg/kg orally.

In tests of platelet behaviour, UK-38,485 inhibited collagen-induced aggregation of human platelet-rich plasma (PRP) *in vitro*, $IC_{50} = 3 \times 10^{-5}M$. Neither UK-38,485 nor aspirin affected aggregation of rabbit PRP to adenosine diphosphate (ADP). However, when a source of PGI_2 synthetase (pig aortic microsomes) was added to UK-38,485-treated rabbit PRP, aggregation by ADP was markedly inhibited. In contrast, this manoeuvre did not expose an anti-aggregatory effect of aspirin.

These results demonstrate inhibition of TxA_2 synthesis and re-direction of arachidonate metabolism towards PGI_2 in blood samples from animals treated with UK-38,485. Selective inhibition of TxA_2 synthesis results in anti-aggregatory activity superior to that seen with cyclo-oxygenase inhibition, provided a source of PGI_2 synthetase is available.

We wish to thank Mrs. Gill Land for her valuable technical contribution to this study.

Moncada, S. & Vane, J.R. (1978) Brit. Med. Bull. 34, 129-133.
Randall, M.J. et al (1981) Thrombosis Res., 23, 145-162.
Tyler, H.M. et al (1981) Lancet, i, 629-632.

EFFECT OF 6-KETO PROSTAGLANDIN E_1 ON SYMPATHETIC NEUROTRANSMISSION IN RAT ANOCOCCYGEUS AND PERFUSED MESENTERIC VASCULAR BED

J. A. Dunning, R. J. Griffiths, P. K. Moore, & J. F. Tucker, Department of Pharmacology, Chelsea College, Manresa Road, London SW3 6LX.

6-keto prostaglandin E_1 (6K PGE_1) is a metabolite of prostacyclin which retains considerable anti-aggregatory and vasodilator activity. In contrast to prostacyclin (PGI_2), 6K PGE_1 potently reduces contractions of the guinea pig vas deferens to field stimulation whilst potentiating the spasmogenic response to exogenous noradrenaline (Griffiths et al, 1982). These results suggest that 6K PGE_1 inhibits release of noradrenaline in this preparation. We have now studied the effect of 6K PGE_1 and PGE_2 on sympathetic transmission in the rat anococcygeus and perfused mesenteric vascular bed.

Anococcygeus muscles from adult male rats were set up as described by Gillespie (1972). Each muscle was suspended in warmed, gassed (95% O_2 : 5% CO_2) Krebs solution and contractions measured isometrically using a Grass FTO3 transducer attached to a Lectromed pen recorder. Field stimulation was carried out via parallel platinum electrodes connected to an SRI square wave stimulator (1 msec ; 100 V). Prostaglandins were incorporated into the Krebs solution. The isolated perfused rat mesenteric arterial preparation was set up as described by McGregor (1965). Changes in perfusion pressure were determined by a Bell & Howell pressure transducer and displayed on a Devices pen recorder. Preparations were either stimulated electrically (15 Hz ; 50 V ; 30 sec) using a bipolar electrode hooked over the exposed artery or by injection of noradrenaline. Prostaglandins were added to the perfusing Krebs solution. 6K PGE_1 (130 nM) decreased motor responses of the field stimulated rat anococcygeus preparation at all frequencies tested (0.25 - 32 Hz). This effect was particularly marked at low frequencies (0.25 - 1Hz). The motor response to exogenous noradrenaline (0.1 - 10 µM) was unaffected by the presence of 6K PGE_1 (130 nM). PGE_2 (56 nM) also inhibited contractions of the field stimulated anococcygeus, but in contrast to 6K PGE_1 potentiated the response to exogenous noradrenaline, particularly at low concentrations (0.01 - 1 µM). Neither yohimbine (10 nM) nor desmethylimipramine (100 nM) affected the inhibitory effect of 6K PGE_1 on motor responses to field stimulation in this tissue.

Intra-arterial injection of noradrenaline (0.2 - 20 µg) or stimulation of the peri-arterial nerve increased perfusion pressure in the rat mesenteric vascular bed. Incorporation of 6K PGE_1 (1.3 µM) into the perfusing Krebs solution produced a small but significant increase in the vasoconstrictor response to nerve stimulation (35.6 ± 2.1 mmHg, n = 6, c.f. 28 ± 4.2 mmHg, n = 6, in the absence of 6K PGE_1) but a much greater potentiation of the effect of injected noradrenaline. For example, the increase in perfusion pressure to 0.5 µg noradrenaline was 71 ± 8.2 mmHg, n = 6, and 139.4 ± 9.2 mmHg, n = 6 in the absence and presence of 6K PGE_1 respectively. PGE_2 (1.4 µM) potentiated the response to nerve stimulation and injected noradrenaline to a similar extent.

The results are consistent with a presynaptic inhibitory effect of 6K PGE_1 in the rat anococcygeus. This effect may also occur in the perfused rat mesenteric bed, although interpretation of results in this preparation are complicated by post-synaptic potentiation.

Gillespie, J.S. (1972) Br. J. Pharmac. 45, 404.
Griffiths, R.J. et al. (1982) This meeting
McGregor, D.D. (1965) J. Physiol. 177, 21.

THE NATRIURETIC ACTIVITY OF $PGF_{2\alpha}$ AND ITS ANALOGUE CLOPROSTENOL IN THE CONSCIOUS RAT

F. A. Ahmed, S. Baharom and J. Haylor, Department of Pharmacology, Sheffield University, Sheffield S10 2TN.

The ability to increase sodium and water excretion is an important renal property of the prostaglandins from which the possible development of a selective natriuretic agent has been proposed (Horton 1979). Of the prostaglandins synthesised in the kidney, $PGF_{2\alpha}$ may possess the most suitable properties from which to develop a diuretic since, unlike PGE_2 or PGI_2 it neither decreases blood pressure nor directly stimulates renin release (Dunn and Hood, 1977; Gerber et al 1981). Information on the natriuretic activity of $PGF_{2\alpha}$ has been obtained mainly from experiments involving its infusion into the renal artery of the dog. However, since the animal model frequently used to screen for diuretic activity is the conscious rat, experiments have been performed to investigate whether the natriuretic activity of $PGF_{2\alpha}$ can be demonstrated following systemic administration in this species. The diuretic activity of the $PGF_{2\alpha}$ - analogue, cloprostenol has also been studied. Unlike $PGF_{2\alpha}$, cloprostenol is not susceptible to metabolism by 15-hydroxyprostaglandin dehydrogenase in the rat in vivo (Bourne et al 1979).

Male rats (300-400g) allowed free access to food and water prior to the experiment were placed in a metabolic cage for a 4 hour period. Urine was collected from each rat at the same time on two consecutive days and assayed for osmolality and a variety of solutes including sodium and potassium. Measurements of fluid and solute excretion obtained on the first day were regarded as the control and were compared to the values obtained after drug treatment on the second day using the paired t-test. Results are expressed as mean $\pm$ s.e.mean. All drugs were dissolved in water and injected subcutaneously in a dose volume of 0.1 ml. Experiments using the drug vehicle alone (n=6) produced no significant change in fluid or solute excretion. The results of experiments with $PGF_{2\alpha}$ and cloprostenol were compared to frusemide 3 mg kg^{-1} (n=6) which produced an increase in urine flow ($P<0.001$) and sodium output ($P<0.001$) while urine osmolality fell ($P<0.001$).

$PGF_{2\alpha}$ 5 $\mu g\ kg^{-1}$ (n=6) and 50 $\mu g\ kg^{-1}$ (n=6) did not alter urine flow or sodium output. Cloprostenol 5 $\mu g\ kg^{-1}$ (n=6) and 50 $\mu g\ kg^{-1}$ (n=6) also produced no change in fluid or solute excretion. However, compared to the control day, $PGF_{2\alpha}$ 500 $\mu g\ kg^{-1}$ (n=6) produced a significant increase in urine formation in the first hour after injection. Urine flow increased from 8.92 ± 1.44 to 40.4 ± 2.02 $\mu l\ min^{-1}$ ($P<0.02$), sodium output increased from 1.29 ± 0.27 to 3.35 ± 0.59 $\mu m\ min^{-1}$ ($P<0.02$), urine osmolality fell from 1301 ± 136 to 501 ± 47 $\mu osm\ ml^{-1}$ ($P<0.01$) while potassium excretion remained unaltered. By the end of the first hour the diuretic effect of $PGF_{2\alpha}$ was essentially complete. Cloprostenol 500 $\mu g\ kg^{-1}$ (n=6) also increased urine flow from 9.33 ± 1.17 to 34.0 ± 4.39 $\mu l\ min^{-1}$ ($P<0.002$), and sodium output from 0.93 ± 0.22 to 4.36 ± 0.36 $\mu m\ min^{-1}$ ($P<0.001$), urine osmolality decreased from 1003 ± 98 to 607 ± 53 $\mu osm\ ml^{-1}$ ($P<0.01$) while potassium excretion remained unaltered in the first hour after injection. Unlike $PGF_{2\alpha}$ however, the natriuretic effect of cloprostenol lasted for at least 4 hours.

We would like to thank ICI and Upjohn for their generous supply of cloprostenol and $PGF_{2\alpha}$ respectively.

Bourne, G. R. et al (1979) Xenobiotica 9(10), 623-631.
Dunn, M. J. and Hood, V. L. (1977) Am. J. Physiol. 233, F162-F184.
Gerber, J. G. et al (1981) Kid. Int. 19, 816-821.
Horton, E. W. (1979) Br. J. Hosp. Med. 22, 260-264.

ANTI-INFLAMMATORY EFFECTS OF AMINOPHYLLINE AND NEOBIPHYLLIN IN GUINEA-PIG SKIN

F.M. Cunningham & L.E. Wood, Department of Clinical Pharmacology, Cardiothoracic Institute, Fulham Road, London, SW3 6HP.

Theophylline (TP) has been shown to inhibit histamine induced permeability changes in the guinea-pig lung in vivo (Persson et al., 1979). It has been suggested that such anti-inflammatory effects may contribute to the therapeutic efficacy of the drug in asthma.

The effects of intravenous (i.v.) administration of Aminophylline (AP) and Neobiphyllin (NP) (a mixed methylxanthine preparation containing TP, mono- and dihydroxy propyl theophylline) on plasma protein extravasation (PPE) in response to intradermal (i.d.) injection of some putative mediators of asthma, have been compared in the guinea-pig skin.

Male Dunkin-Hartley guinea-pigs were injected i.v. with 0.5 ml of Evan's blue dye containing 125-I human serum albumin, simultaneously with 2.5 ml/kg phosphate buffered saline (PBS), AP (60 mg/kg) or NP (100 mg/kg). The dose of AP was calculated to give a plasma concentration of 10-70 ug/ml TP (Madsen and Ribel, 1981). The dose of NP administered was calculated to be equi-effective with AP, by correcting for the in vitro potencies of the constituent drugs on smooth muscle relaxation (Svedmyr, 1977). Six treatments (i.d. injections of PBS, two doses of platelet activating factor (PAF-acether), two doses of leukotriene D4 (LTD4) plus prostaglandin E (PGE2) and histamine (His)) were allocated in duplicate to the skin sites of shaved animals. After 30 mins the animals were killed, bled and then skinned. The lesions were punched out and the 125-I content in skin sites and 1 ml blood samples counted. The plasma protein extravasation (expressed as ul equivalents of whole blood) in treatment groups was compared to controls (Table 1):

Table 1 Effect of AP and NP on plasma protein extravasation

Mediator	Plasma protein extravasation (ul equivalents of whole blood)		
	Control (n=6)	AP (n=5)	NP (n=8)
PAF-acether (0.3 ng)	33.4 + 5	25.4 + 5	*17.9 + 3
PAF-acether (3 ng)	104 + 8	77.0 +10	*59.6 + 8
LTD4 (1 ng) + PGE2 (100 ng)	11.7 + 2	16.2 + 3	9.3 + 3
LTD4 (10 ng) + PGE2 (100 ng)	47.7 + 2	39.9 + 5	32.0 + 7
His (0.5 ug)	71.0 + 6	60.0 +12	*43.7 + 8

Results are expressed as mean + s.e. mean. * $p<0.05$.

Plasma protein extravasation in response to i.d. injection of PAF-acether, LTD4 (10 ng/site) plus PGE2 and His was reduced, following i.v. injection of NP and AP. The reduction of PAF-acether and His responses by NP was statistically significant. These results suggest that the anti-permeability properties of the two methylxanthine preparations may, at least in part, explain the effectiveness of the drugs in the therapy of obstructive airway disease.

Madsen, S.M. & Ribel, U. (1981). Acta Pharmacol.et toxicol. 48, 1-7.
Persson, E.G.A., Ekman, M. & Erjefalt, I. (1979) Acta Pharmacol. et toxicol. 44, 216-220.
Svedmyr, N. (1977). Scand. J. Resp. Dis. Suppl. 101, 13-24.

PARADOXICAL EFFECTS OF INDOMETHACIN ON 4-HOUR AND 24-HOUR CARRAGEENAN-INDUCED PLEURISIES

D Bradshaw, P H Franz, S J Greenham. Department of Pharmacology, Roche Products Ltd., Welwyn Garden City, Hertfordshire AL7 3AY.

Carrageenan-induced pleurisy is a useful model for quantitating both the oedematous and cellular aspects of an inflammatory response. Numerous reports have documented an inhibitory effect of indomethacin on both these parameters for the early phase (up to 6h) of the response. However, relatively few studies have considered the effects of indomethacin on the later stages of the response and those which did produced somewhat contradictory results (Ackerman et al., 1980; Ammendola et al., 1975; Meacock et al., 1979; Schiantarelli, et al; 1978). We have compared the effects of indomethacin on the pleural inflammatory response to different types of carrageenan at 4h and 24h after its injection.

Female Alderley Park strain rats weighing 220-250g were injected intrapleurally under ether anaesthesia with 0.2 ml of a 1% carrageenan suspension in saline. Indomethacin was administered orally 1h before carrageenan (4h pleurisy) or 1h before and 5h after (24h pleurisy) carrageenan. Pleural exudates were collected from animals killed 4h or 24h after injection and the pleural cavity was washed out with 2 ml of phosphate-buffered saline. The exudate volume was recorded and cell counts were determined using a haemocytometer or Coulter automatic cell counter. Differential cell counts were performed for the 24h pleurisy.

Using Viscarin carrageenan (Marine Colloids), indomethacin produced a dose-related inhibition of exudate volume at 4h after injection. This inhibition reached statistical significance at doses of 10 $mg.kg^{-1}$ (58%, $P < 0.001$) and 30 $mg.kg^{-1}$ (67%, $P < 0.001$). Significant inhibition of cell accumulation (up to 43%, $P<0.001$) was observed at 3, 10 and 30 $mg.kg^{-1}$. When lambda-carrageenan (Sigma) was used as the irritant, indomethacin (3 and 10 $mg.kg^{-1}$) inhibited both exudate volume (up to 57%, $P < 0.001$) and cell accumulation (up to 33%, $P < 0.001$).

At 24h after injection of Viscarin, indomethacin (total dose 10 $mg.kg^{-1}$) had no significant effect on either of the two parameters measured. However, when lambda-carrageenan was used, indomethacin (3, 10 and 30 $mg.kg^{-1}$) - treated animals showed a dramatic (up to 8-fold) increase in exudate volume compared with vehicle-treated controls. At 10 and 30 $mg.kg^{-1}$, indomethacin selectively inhibited (up to 58%, $P < 0.001$) the accumulation of mononuclear cells with no effect on polymorphonuclear leucocytes.

These results indicate a clear difference in the effect of indomethacin on 4h and 24h pleurisies and re-affirm previous reports that the various types of carrageenan induce different types of inflammation. The most remarkable effect observed was the production by indomethacin of a large increase in exudate volume for the lambda-carrageenan pleurisy. Preliminary experiments suggest that this may be due to an inhibition, by indomethacin, of the re-absorption of pleural fluid. It may be that this is apparent only for the lambda carrageenan-induced inflammatory response owing to its shorter time-course compared with that induced by Viscarin.

Ackerman, N. et al (1980) J. Pharm. exp. Ther. 215, 588-595.
Ammendola, G. et al (1975) Agents Actions 5, 250-255.
Meacock, S.C.R. et al, (1979) Eur. J. Rheumatol. Inflamm. 3, 23-28.
Schiantarelli, P.W. et al (1978) Arzneim. Forsch. 28, 1146.

THE MECHANISM OF ACTION OF SOME INHIBITORS OF 15-LIPOXYGENASE

W.P. Kingston, & A. Ritchie-Smith, Department of Pharmacology, Life Sciences Building, Sunderland Polytechnic, Sunderland, SR1 3SD.

15-Lipoxygenase from soybean has previously been reported to provide a rapid assay for lipoxygenase inhibitors (Kingston 1981). In the present study we have used 15-lipoxygenase to investigate the mechanism of inhibition of a number of anti-inflammatory drugs and also the agents Nordihydroguaiaretic acid (NGA) and 1-phenyl-3-pyrazolidone (1-P-3-P).

Lipoxygenase activity was determined polarographically with an oxygen electrode. Incubations were performed at 37°C in 100mM phosphate buffer pH7.5 containing 240μM arachidonic acid and 80μg soybean lipoxygenase. The mixture was stirred gently and the change in oxygen saturation of the mixture was recorded for 3 min. The nature of enzyme inhibition and the inhibitor constant (K_i) were determined as described by Tipton (1980). Results represent the mean ± s.e.mean for 6 determinations performed on 2 occasions. Inhibition of 15-lipoxygenase was produced by several agents (Table 1). Aspirin and piroxicam had no effect.

Table 1. Inhibition of 15-lipoxygenase.

	K_i	Nature of Inhibition
NGA	1.58 ± 0.92μM	competitive
BW 755C	2.21 ± 0.95μM	irreversible
1-P-3-P	8.82 ± 0.16μM	irreversible
Indomethacin	1.59 ± 0.92mM	irreversible
Benoxaprofen	8.3 ± 0.80mM	competitive
Fenoprofen	9.0 ± 0.18mM	competitive
Flurbiprofen	17.0 ± 0.91mM	competitive
Ketoprofen	18.0 ± 0.81mM	competitive
Ibuprofen	34.0 ± 0.43mM	competitive

With the exception of BW 755C, high concentrations of anti-inflammatory agents were required to inhibit 15-lipoxygenase. These concentrations were considerably higher than those reported for inhibition of cyclo-oxygenase (Flower, 1974). It is possible that at high concentrations these agents may have the ability to act as dual inhibitors of cyclo-oxygenase and lipoxygenase, whereas at low concentrations they selectively inhibit cyclo-oxygenase. The proprionic acid derivatives (benoxaprofen, fenoprofen, flurbiprofen, ketoprofen and ibuprofen) all acted as competitive inhibitors of 15-lipoxygenase suggesting a common mechanism of action. Indomethacin was observed to act as an irreversible inhibitor of 15-lipoxygenase. It has previously been observed to irreversibly inhibit cyclo-oxygenase (Flower, 1974).

15-Lipoxygenase from soybean thus provides a rapid assay for the potency and mechanism of action of lipoxygenase inhibitors.

Flower, R.J. (1974). Pharmac. Rev., 26, 33.
Kingston, W.P. (1981). Br.J.Pharmac., 74, 919P.
Tipton, K.F. (1980). In Enzyme Inhibitors as Drugs ed. by M. Sandler, Macmillan press. P1.

THE RETENTION OF FREE AND LIPOSOME-ENTRAPPED METHOTREXATE IN ARTHRITIC JOINTS

W.C. Foong & K.L. Green, School of Pharmacy, Portsmouth Polytechnic, Portsmouth, PO1 2DT.

Dingle et al (1978) have shown that the entrapment of corticosteroids in liposomes greatly enhances their anti-inflammatory effect when injected into arthritic joints. We have been investigating the possibility of entrapping cytotoxic drugs in liposomes for intra-articular injection with the objective of effecting a chemical synovectomy. Methotrexate (MTX) has been administered intra-articularly to control the synovitis in arthritic joints, but with disappointing results, probably because the drug is rapidly cleared from injected joints (Bird et al, 1977; Wigginton et al, 1980).

Multilamellar liposomes (average diameter 1μ) containing ^{3}H-MTX were prepared from egg phosphatidylcholine (PC), cholesterol (CH) and dicetylphosphate (DCP) in a molar ratio of 5:5:1. An experimental arthritis was induced in the knee joints of rabbits using the procedure described by Dumonde & Glynn (1962), but using ovalbumin as the antigen. Rabbits with normal knee joints, or with joints in which arthritis had been induced 3 weeks previously, were injected intra-articularly with either free or liposome entrapped ^{3}H-MTX. Blood samples were collected at regular intervals and the radioactivity of the plasma measured.

Peak plasma levels of ^{3}H-MTX occurred 1 h after injection of either the free or liposome entrapped drug, and represented 6.80 ± 0.24% and 0.91 ± 0.09% (n = 4) respectively of the total amount injected into arthritic joints. The rabbits were killed 4 h after injection and radioactivity in the injected joint and various other tissues determined. At 4 h the retention of ^{3}H-MTX in the injected joint was more than 12 times greater when administered in liposome entrapped form than as the free drug (Table 1).

Table 1. Recovery of ^{3}H-MTX from joints 4 h after injection expressed as a percentage of the amount injected (mean ± s.e. mean)

	Normal joint Free MTX	Arthritic joint Free MTX	Arthritic joint Liposomal MTX
Synovial fluid	3.45 ± 0.43	3.18 ± 0.55	45.83 ± 8.70
Synovial membrane	0.05 ± 0.03	0.23 ± 0.08	1.39 ± 0.42
Total recovered from injected joint	3.72 ± 0.45 n = 3	3.74 ± 0.73 n = 4	48.15 ± 8.81 n = 4

Wigginton et al (1980) have suggested that adequate concentrations of MTX to inhibit synovial proliferation could be maintained in arthritic joints by giving multiple injections of the drug, systemic toxicity being prevented by the oral administration of leucovorin. Preliminary results reported here indicate that the intra-articular injection of liposome entrapped MTX might achieve the same objective without the necessity of multiple injections or leucovorin prophylaxis.

This work was supported by a grant from the Nuffield Foundation. We thank Lederle for generous supplies of methotrexate.

Bird, H.A. et al (1977) Curr.Med.Res.Opin. 5, 141-146
Dingle, J.T. et al (1978) Nature 271, 372-373
Dumonde, D.C. & Glynn, L.E. (1962) Br.J.exp.Path. 43, 373-383
Wigginton, S.M. et al (1980) Arth.Rheum. 23, 119-122

N,N-DIMETHYLFORMAMIDE, A COMPOUND WITH SMOOTH MUSCLE RELAXANT PROPERTIES, INHIBITS ENERGY METABOLISM IN ISOLATED MITOCHONDRIA

G.S. Lovett and A.J. Sweetman, School of Health and Applied Sciences, Leeds Polytechnic, Leeds LS1 3HE.

The non-polar solvent, N,N-dimethylformamide (DMF), has been shown to relax smooth muscle preparations, such as the coaxially stimulated ileum and the taenia caecum of the guinea-pig (Nicholson et al, 1981). Since a number of smooth muscle relaxant drugs have been found to interfere with mitochondrial energy production (Foster et al, 1978), we have examined the effect of DMF on this process, using mitochondria isolated from guinea-pig liver as a model system.

Mitochondria were isolated from the livers of guinea-pigs by homogenisation and differential centrifugation. ADP-stimulated respiration was used as a measure of the ATP synthesising ability of isolated mitochondria. Respiration stimulated by 2,4-dinitrophenol was chosen to determine the effect of DMF on the activity of this control drug. Both activities were measured using an oxygen electrode.

The low rate of endogenous respiration that occurred in the absence of the phosphate acceptor (ADP) was unaffected by DMF (4 - 400 mM : 0.03 - 3% v/v). This finding ruled out the possibility of an uncoupling action for the solvent. When ADP was present, DMF (4 - 400 mM) elicited a concentration-dependent inhibition of ADP-stimulated respiration. A similar pattern of activity was seen whether succinate (IC50 = 189.6 $\pm$ 25.2 mM; n = 5), or glutamate plus malate (IC50 = 234.4 $\pm$ 37.6 mM; n = 5) was used as substrate. These two results were not significantly different. The inhibition was not overcome by the addition of uncoupling concentrations of 2,4-dinitrophenol, thus eliminating the possibility of a direct action on the ATP synthetase enzyme system. A more likely explanation is that DMF interacts with a component of the terminal portion of the respiratory chain, since both succinate and glutamate plus malate oxidation were inhibited equally.

To determine whether this inhibitory action of DMF could interfere with analysis of the activity of other compounds, we examined the effect of DMF on respiration stimulated by 2,4-dinitrophenol. 2,4-Dinitrophenol (15 µM) produced a six-fold enhancement of succinate oxidation, to give a maximal rate of 41.2 $\pm$ 3.2 ngatom oxygen/min/mg mitochondrial protein (n = 5). DMF (400 mM) reduced this rate to 10.1 $\pm$ 3.8 ngatom oxygen/min/mg mitochondrial protein (n = 5). IC50 values were: succinate (197.6 $\pm$ 36.8 mM; n = 5) and glutamate plus malate (187.2 $\pm$ 47.2; n = 5)

We conclude that the non-polar solvent DMF inhibits mitochondrial ATP synthesis; an action that could explain the smooth muscle relaxing properties of the compound, and which could seriously affect the assessment of drug-mitochondria interactions, if DMF was employed as the solubilising agent for the drug.

Foster, H.E. et al (1978) Br. J. Pharmac. 63, 309-314
Nicholson, J. et al (1981) Br. J. Pharmac. 74, 300-301P

STUDIES ON THE INTERACTION OF THE STEROID ANAESTHETIC ALTHESIN AND THE BARBITURATE METHOHEXITONE IN THE RAT

R. Cooksey and A. Livingston. Department of Pharmacology, Medical School, Bristol BS8 1TD.

It has been reported (Archer, Richards & White, 1977; Richards & White, 1981) that there is a synergism between the steroid anaesthetic Althesin (a mixture of alphaxalone and alphadolone) and the barbiturate methohexitone, which results in a prolongation of sleeping time in rats. It was suggested that this effect was most likely to be the result of the drugs acting at different sites.

We have examined these effects in groups of twelve male Wistar rats, body weight 300-450g, by constructing dose response curves for the sleeping times for various doses of Althesin, ranging from 15 to 30 mg/kg, and similarly for methohexitone, with doses ranging from 35 to 80 mg/kg I.P.

Over these dose ranges, there was an approximately linear relationship between effect and dose for both agents. From these graphs a dose was calculated for each agent which would give a particular sleeping time, and a combination consisting of a half dose of each anaesthetic was given for each predicted sleeping time ranging from five to forty minutes. In every case there was an increase in the actual sleeping time over the predicted time, ranging from 40 to 75%.

To examine the plasma levels of the drugs at various times after injection, groups of four male rats, body weight 400-500g, were injected either with a dose of 33.5 mg/kg Althesin I.P., or 86.6 mg/kg methohexitone I.P., both of which would give a sleeping time of about 25 minutes, or with a combined dose of 17.0 mg/kg Althesin and 43.5 mg/kg methohexitone. Groups of animals were killed by decapitation at 1, 5, 10, 25 and 50 minutes after injection and heparinised blood samples collected. Plasma was obtained by centrifugation and stored frozen until assay by gas-liquid chromatography.

Clearance curves for methohexitone and alphaxalone (the major constituent of Althesin) were constructed and it appeared that the clearance of alphaxalone was very similar whether the drug was given alone or in combination, namely at each time point after the first reading the half dose gave almost exactly half (42-48%) the plasma concentration that the full dose did.

In the case of methohexitone, however, the pattern was not the same, with plasma levels for the half dose (in combination with Althesin) being raised to approximately three quarters (72-80%) of the full dose plasma level at 5, 25 and 50 minutes.

These results suggest that there is a lower rate of clearance of plasma methohexitone in the presence of Althesin and that the prolongation of sleeping time seen when the drugs are used in combination might be accounted for by the prolongation of methohexitone levels in the plasma.

Archer, E.R., Richard, C.D. & White, A.E. (1977) Br.J.Pharmac. 59: 508P.

Richards, C.D. & White, A.E. (1981) Br.J.Pharmac. 74: 161-170.

EFFECT OF N-(PHOSPHONACETYL)-L-ASPARTATE ON THE FLUX THROUGH THE DE NOVO PYRIMIDINE BIOSYNTHETIC PATHWAY OF MOUSE TUMORS IN VIVO

L.A. Anderson, R.L. Cysyk, A. Monks*, and J. Strong, LCP, DCT, National Cancer Institute, Bethesda, MD, 20205, U.S.A. (Introduced by A. Richens)

N-(phosphonacetyl)-L-aspartate (PALA) is an analogue of the transition state intermediate of the reaction catalyzed by the enzyme aspartate transcarbamylase and has been shown to inhibit activity of this enzyme in vitro (Collins and Stark, 1971) and in tissues isolated from animals treated with PALA (Yoshida et al, 1974). PALA is active against slow growing solid tumors in mice, including Lewis Lung carcinoma, but is ineffective against most rapidly growing tumors such as L1210 leukemia (Johnson et al, 1976). An understanding of the spectrum of antitumor activity of PALA requires studies on the flux through the pyrimidine biosynthetic pathway in vivo. This in vivo flux cannot be measured with radioactive precursors due to lack of adequate incorporation into the acid soluble uracil nucleotide pool (Σuracil) of tissues in situ. Therefore, we have developed a GC/MS technique which enables measurement of the incorporation of stable label from $^{13}CO_2$ into Σuracil of mouse tumors in vivo. In each experiment, control (n=3) and PALA treated (n=4) mice with tumors in situ were exposed to 10% $^{13}CO_2$ for 20 min followed by rapid excision and freezing of the tumors. Quantitation of Σuracil was by HPLC analysis and the $^{13}C/^{12}C$ ratio was measured by electron impact mass spectroscopy.

Table 1: The inhibitory effect of an optimal therapeutic dose of PALA (400mg/kg) on the incorporation of ^{13}C into Σuracil of L1210 leukemia (s.c. or i.p.) or Lewis Lung carcinoma (LL Ca) in BDF_1 mice. NS = no significant difference between treated and non-treated animals.

TUMOR	PRETREATMENT TIME	%DECREASE IN ΣURACIL + SD	%INHIBITION OF ^{13}C INCORPORATION + SD
L1210 i.p.	1	66 ± 22	93 ± 6
L1210 s.c.	1	NS	93 ± 11
LL Ca	1	NS	88 ± 19
L1210 i.p.	12	75 ± 11	63 ± 33
L1210 s.c.	12	43 ± 6	NS
L1210 i.p.	24	17 ± 3	32 ± 9
L1210 s.c.	24	43 ± 10	NS
LL Ca	24	NS	83 ± 16
L1210 i.p.	48	NS	NS
L1210 s.c.	48	NS	NS
LL Ca	48	47 ± 15	93 ± 13

The results show that a single dose of PALA inhibits the flux through the de novo pyrimidine biosynthetic pathway of L1210 (PALA resistant) and LL Ca (PALA sensitive) tumors in vivo. This effect occurs within 1h of the dose for all tumors and persists for 48h in LL Ca. Recovery occurred within 12h for the L1210 s.c. tumors and within 12-24h for the L1210 i.p. tumors. These studies indicate that the resistance of L1210 tumors to PALA could be due to the capacity of this tumor to rapidly recover from inhibition of flux through its de novo biosynthetic pathway in vivo.

Collins, K.D. & Stark, G.R. (1971) J. Biol. Chem. 246, 6599P
Johnson, R.K. et al (1976) Cancer Res. 36, 2720P
Yoshida, T. et al (1974) J. Biol. Chem. 249, 6951P

SEX-LINKED DIFFERENCES IN THE METABOLISM AND IRREVERSIBLE BINDING OF (^{3}H)-ETHYNYLOESTRADIOL IN THE RAT

P.S. Grabowski, J.L. Maggs and B.K. Park, Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX.

17α-Ethynyloestradiol (EE_2) undergoes extensive 2-hydroxylation *in vitro* and *in vivo* (Breckenridge et al., 1981; Brown et al., 1981). Further oxidation of the resulting catechol (2-OHEE_2) produces a chemically reactive quinone or semiquinone which reacts with proteins and peptides (Kappus and Bolt, 1973). As part of our studies of factors which influence the formation of EE_2 reactive metabolite(s) *in vivo*, we have investigated the metabolism and irreversible binding of [^{3}H]EE_2 in male and female Wistar rats (250-300g).

Bile was collected for 3h after i.v. administration of [^{3}H]EE_2 (5μg kg^{-1}; 20μCi kg^{-1}). The biliary conjugates were hydrolysed with a mixture of β-glucuronidase and arylsulphatase, and the deconjugated metabolites analysed by hplc (Maggs and Park, 1982). The amount of ^{3}H irreversibly bound to hepatic soluble and microsomal protein was measured (Brown et al., 1981). In addition, the tissue distribution of total ^{3}H was determined at 3h.

A significantly greater percentage of the dose of [^{3}H]EE_2 was irreversibly bound to hepatic proteins from females at 3h ($\bar{x}$ ± s.d., n = 4 or 5):

Microsomal protein (total):- females 0.56 ± 0.10%; males 0.24 ± 0.07% ($p < 0.001$)

Soluble protein (total):- females 1.37 ± 0.49%; males 0.41 ± 0.13% ($p < 0.01$).

This was despite the fact that the accumulation of total ^{3}H was significantly ($p < 0.001$) greater in the male liver (male: 17 ± 4%; female 8 ± 2% of dose).

Table 1 Deconjugated Biliary Metabolites of [^{3}H]EE_2 from Male and Female Rats

Sex	% ^{3}H extracted into ether	Unknown	Deconjugated ^{3}H metabolites ($\bar{x}$ % ± s.d., n=4)† 16-OHEE$_2$	2-OHEE$_2$	EE$_2$	2-MeOEE$_2$	2-OHME
Male	72 ± 4	18 ± 7	5 ± 1	20 ± 6	14 ± 1	25 ± 7	5 ± 1
Female	61 ± 4*	7 ± 1††	3 ± 1	43 ± 1***	10 ± 1**	20 ± 3	8 ± 1**

† = 2-Hydroxymestranol; †† $p < 0.05$; * $p < 0.01$; ** $p < 0.005$; *** $p < 0.001$.

There were significant sex-linked differences in the proportions of most of the deconjugated biliary metabolites (Table 1). It appears that the fraction of 2-OHEE_2 that underwent catechol O-methylation was lower in females than in males.

These data suggest that catechol O-methylation may play an important role in determining the extent of irreversible binding by regulating the availability of reactive-metabolite precursor. Additionally, they demonstrate that sex-linked differences in drug metabolism are not confined to Phase I biotransformations.

This work was supported by the Wellcome Trust.

Breckenridge, A.M. et al., (1981). Br. J. Pharmacol. 74, 240P.
Brown, I.C. et al., (1981). Br. J. Pharmacol. 74, 987P.
Maggs, J.L. & Park, B.K., (1982). Br. J. Pharmacol. in press.
Kappus, H. et al., (1973). Steroids 22, 203.

THE EFFECT OF PHENOBARBITONE ON THE PHARMACOKINETICS AND PHARMACODYNAMICS OF VITAMIN K IN THE RABBIT

A.M. Breckenridge, B.K. Park & A.C. Wilson, Department of Pharmacology and Therapeutics, University of Liverpool, Liverpool L69 3BX

Mountain et al., (1970) reported that neonates born to epileptics on long term anticonvulsant therapy, including phenobarbitone or phenytoin, often have a blood coagulation defect resembling vitamin K_1 deficiency. Since chronic enzyme induction may enhance metabolic inactivation of a number of endogenous compounds, for example vitamin D, we examined the effect of phenobarbitone enzyme induction on the pharmacokinetics and pharmacodynamics of vitamin K_1 in the male NZ white rabbit.

Animals (2.5 - 3.0kg), first given either phenobarbitone (40 mg kg^{-1} i.p.) or saline for 4 days, received vitamin K_1 (2.2μmol.kg^{-1},Konakion®) into the marginal ear vein and blood samples were taken over 6h. Vitamin K_1 and vitamin $K_1$2,3-epoxide were measured in hexane extracts of plasma by normal phase h.p.l.c. using a Partisil PXS column (25cm x 4.5mm; particle diameter 10μm) with acetonitrile-hexane (1:500 v/v; flow rate 2ml/min^{-1}) as eluent. The pharmacological response to vitamin K_1 was determined in rabbits anticoagulated with 1mg kg^{-1} brodifacoum (prothrombin complex activity or P.C.A. < 30%) as described by Park et al., (1980).

Table 1 Effect of phenobarbitone on the pharmacokinetics of vitamin K_1 in the rabbit

	Control	Phenobarbitone	
Vitamin K_1 $t_{\frac{1}{2}}\alpha$ (h)	0.18 ± 0.01	0.14 ± 0.02	
Vitamin K_1 $t_{\frac{1}{2}}\beta$ (h)	2.08 ± 0.56	0.99 ± 0.30	**
Vitamin K_1 A.U.C. (nmol.ml^{-1} h)	60.32 ± 2.40	46.50 ± 8.01	*
Vitamin K_1 V.D. (L. kg^{-1})	0.098 ± 0.006	0.068 ± 0.018	*
K_1 Plasma Clearance (ml min^{-1}.kg^{-1})	0.609 ± 0.023	0.807 ± 0.142	*

Values are expressed as mean (n=4) ± S.D.* $p < 0.05$; ** $p < 0.01$

Phenobarbitone did not effect the vitamin K_1 2,3-epoxide : vitamin K_1 ratio indicating there was no interference with the physiologically important vitamin K_1 - epoxide cycle. Control plasma half-lives for the fast ($t_{\frac{1}{2}}\alpha$) and slow ($t_{\frac{1}{2}}\beta$) components of the plasma concentration-time curve (Table 1) are similar to values obtained using a tracer (1.59 nmol. kg^{-1}) dose of ^3H vitamin K_1 (Park et al., 1980). Phenobarbitone treatment significantly reduced $t_{\frac{1}{2}}\beta$, the area under the plasma concentration-time curve (A.U.C.) and the volume of distribution. However, the plasma half-life for the disappearance of vitamin K_1 2,3-epoxide was unchanged. Phenobarbitone did not alter the rate, duration of recovery or subsequent decline of P.C.A. after vitamin K_1 administration to anticoagulated animals.

These results show that phenobarbitone increases the plasma clearance of vitamin K_1. However, phenobarbitone has no apparent effect upon either the hepatic metabolism or the biliary excretion of vitamin K_1 in the rat (Leck, 1982). Nevertheless, phenobarbitone clearly alters the disposition of vitamin K_1 in the rabbit, although the mechanism of the interaction remains to be determined.

This work was supported by grants from the British Heart Foundation and the Mersey Regional Health Authority.

Leck, J.B. (1982) Ph.D. Thesis, University of Liverpool.
Mountain, K.R. (1980) Lancet, i, 265.
Park, B.K. et al., (1980) In: Vitamin K metabolism and vitamin K dependent proteins, pp 348 University Park Press, Baltimore.

THE EFFECT OF FRUSEMIDE ON (^{86}Rb) UPTAKE INTO RAT LIVER SLICES INCUBATED IN VITRO

B.E. Argent[1], Frances C. Hirst & E. Poong[1], (introduced by B.A. Hemsworth), Department of Pharmacology, Sunderland Polytechnic, Sunderland, SR1 3SD and [1]Department of Physiological Sciences, Medical School, University of Newcastle Upon Tyne, Newcastle Upon Tyne, NE1 7RU.

Hypokalaemia is often associated with long term diuretic therapy. Since a concurrent reduction in total body potassium is not always observed (Anderson et al, 1971; Dargie et al, 1974), the possibility exists that diuretics may cause a redistribution of potassium between extracellular and intracellular compartments. In this study, we have investigated this possibility by examining the effects of furosemide on ^{86}Rb uptake (as a tracer for potassium) into the intracellular pool of rat liver.

Slices, weighing approximately 20mg, were prepared from the livers of 60-70g rats of either sex. The slices were incubated at 37°C in a Krebs-Ringer bicarbonate medium (continuously gassed with 95% O_2 / 5% CO_2) containing approximately 20μCi/ml ^{86}RbCl. At time intervals up to 120min, the slices were removed from the medium gently blotted on damp filter paper and then washed for 30 min at 0°C in a K^+-free medium containing ouabain (1mM). This procedure removes extracellular ^{86}Rb, but preserves the intracellular pool. Subsequently the slices were weighed and the ^{86}Rb activity associated with the tissue determined. Under all experimental conditions the rate of ^{86}Rb uptake was linear with time over the first 60 min of incubation.

In control experiments the ^{86}Rb uptake after 60 min into the intracellular pool of the slices was 20.3 ± 1.6μmoles/g wet wt. (mean ± s.e., n = 8). Ouabain (1mM) reduced this value to 9.0 ± 1.8μmoles/gwet wt. (n=8, $P<0.05$) whereas furosemide (10μM) increased ^{86}Rb uptake to 30.8 ± 2.2μmoles/g wet wt (n=8, $P<0.05$). Increasing the furosemide concentration to 1mM had no further effect on ^{86}Rb uptake. In the presence of ouabain (1mM) the stimulatory effect of furosemide (10μM) on ^{86}Rb uptake was abolished.

In calculating results, we have assumed that as Rb^+ and K^+ are transported in the same manner (Rendi & Uhr, 1964), alterations in ^{86}Rb uptake accurately reflect changes in K^+ uptake. These data suggest that furosemide enhances K^+ uptake into liver cells by a mechanism that involves stimulation of Na, K-ATPase in the plasma membrane.

Anderson, J. et al (1971). J.Med., 40, 541.
Dargie, H.J. et al (1974). Br.Med.J., 4, 316.
Rendi, R. & Uhr, M.C. (1964). Biochim.Biophys.Acta, 89, 520.

A SIMPLE METHOD OF QUANTIFYING IONOPHORESIS OR PRESSURE EJECTION OF CATECHOLAMINES FROM CARBON FIBRE MICROELECTRODES

Z.L.KRUK & J.A.STAMFORD, Department of Pharmacology, The London Hospital Medical College, Turner Street, London EA1 2AD, U.K.

During experiments using carbonfibre microelectrodes for quantification of catecholamine ionophoresis (1,2,3) or pressure ejection, it became apparent to us that it was possible to simplify the apparatus, and thus make the methodology available in any neuroscience laboratory equiped for electrical recording in the CNS. The technique depends on the measurement of the current flowing in the carbonfibre microelectrode during electroxidation or reduction of catecholamine adsorbed on the surface of the electrode. Whereas in the previously published method (1) a gated waveform was always used, we have found that a good estimate of the concentration of the electroactive species can be obtained using an ungated driving voltage ramp. The obvious advantage of using the gated waveform is that it is possible to measure unit activity using the same carbonfibre microelectrode in the intervals between electrochemical sweeps.(4). Frequency of making measurements of concentration is reduced in the present method, but this does not constitute a major drawback once steady state ionophoresis has been established. The electrochemical signal is monitored on a digital storage oscilloscope, and recorded on a FM tape recorder. The sensitivity of the technique is better than 1.10^{-6} M. catecholamine.

Figure 1. Electrochemical oxidation (O) and reduction (R) signals to ionophoresed dopamine. The smallest trace was obtained during 40 nA retain current, and the other three traces during steady state 5,10 and 20 nA eject current. The driving voltage ramp (not shown), was a symetrical ramp about 0 volts, moving from +1 volt to -1 volt at 20Hz.

1.Armstrong-James,M, Fox,K, Kruk,Z.L, & Millar,J.(1981).J.Neurosci.Methods.4 385-406.
2.Armstrong-James,M, Millar,J, & Kruk,Z.L.(1980). Nature 288 181-183
3.Millar,J, Armstrong-James, M, & Kruk, Z.L.(1981). Brain Res 205 419-424
4.Armstrong-James,M.(1982). Proc. 1st.British Electrochemical Detection Meeting, Nottingham. C5.

TWO METHODS FOR THE DETERMINATION OF HISTIDINE DECARBOXYLASE ACTIVITY

D.J. Keeling, I.R. Smith[1], K.F. Tipton, Department of Biochemistry, Trinity College, Dublin, Eire and [1]Smith Kline and French Research Limited, The Frythe, Welwyn, Hertfordshire, England.

The low levels of histidine decarboxylase activity in many tissues necessitate assay methods of high sensitivity. Two such methods were developed.

The first (direct) assay method was an extension of the radioisotopic method of Baudry et al, (1973) incorporating an extraction into scintillant containing a liquid cation-exchanger (McCaman et al, 1972). The inclusion of this step improved the separation of the enzymatically produced {^{3}H} histamine from the {^{3}H} histidine used as substrate and doubled the sensitivity of this method. The direct assay permitted the rapid performance of large numbers of assays (more than 100 per day).

A coupled assay method for measuring histidine decarboxylase has already been described (Keeling & Smith, 1979). This method has now been extensively modified. The enzymatically produced {^{3}H} histamine was converted in situ to {^{3}H}N^{τ}-methylhistamine using the enzyme histamine N-methyltransferase and S-adenosylmethionine. The {^{3}H}N^{τ}-methylhistamine was then isolated by chloroform extraction, ion-exchange chromatography and liquid cation-exchanger extraction. This method proved extremely sensitive, detecting the conversion of 0.0007% of substrate to product. Samples containing as little as 0.14 fmol/min/ml histidine decarboxylase activity (assayed at 1 μM {^{3}H} histidine) could be measured using the coupled assay.

Baudry, M. et al (1973) J. Neurochem. 21, 1301 - 1309.
Keeling, D. J. & Smith, I. R. (1979) Biochem. Soc. Trans. 7, 672 - 673.
McCaman, M. W. et al (1972) Analyt. Biochem. 45, 242 - 252.

MEASUREMENT OF HISTIDINE DECARBOXYLASE ACTIVITY IN THE RAT AND KITTEN ISOLATED GASTRIC MUCOSA USING A NEW SPECIFIC ASSAY

D.J. Keeling, M.E. Parsons, C.A. Price, I.R. Smith and S.K. Tingley, Smith Kline and French Research Limited, The Frythe, Welwyn, Hertfordshire, AL6 9AR.

We have previously shown that pentagastrin increased histidine decarboxylase (HDC) activity in the rat isolated gastric mucosa using the CO_2 trap assay described by Hakanson et al (1974). Metiamide inhibited this effect but not in a dose dependent manner (Bunce et al, 1981). The aim of this study was to investigate HDC activation in the kitten isolated gastric mucosa and compare this with HDC activation in the rat where histamine metabolism is atypical.
Since levels of HDC in the kitten gastric mucosa has been reported to be low (Lorenz et al. 1969) we have used a new, more sensitive HDC assay described by Keeling et al (1982). This assay uses L-{2,5-^{3}H} histidine as the substrate and histamine methyltransferase (HMT) as a coupling enzyme, (^{3}H) methylhistamine being the product. Unlike the CO_2 trap assay it specifically measured the formation of histamine. The gastric mucosae of male Wistar rats, (approx. 200 g) and kittens, (700-800 g) were separated from the muscle layers using the 'blistering' technique described by Forte et al, (1975). Paired mucosae were mounted in organ baths according to the method of Main and Pearce, (1978) and the mucosal surface superfused with unbuffered Krebs-Henseleit solution (1 ml/min). The acid output was measured as described by Bunce and Parsons, (1976). Pentagastrin was added to the serosal solution and after a 30 min contact period the mucosae were removed from the organ baths, blotted dry, weighed and homogenized in ice cold 0.1M Tris HCl buffer (pH 7.2) to a final concentration of 100 mg/ml for rat and 200 mg/ml for kitten mucosa.
Measurement of HDC in rat tissue was complicated by inhibition of HMT. This inhibition was not removed by addition of the diamine oxidase (DAO) inhibitor aminoguanidine (10 μM), suggesting no interference with HMT by endogenous DAO, the major histamine metabolising enzyme in rat mucosal tissue (Kobayashi and Ivy, 1959). The inhibition of HMT could be overcome by increasing the amount of coupling enzyme. This suggests the presence of an endogenous inhibitor of HMT in the rat gastric mucosa.

In the rat isolated gastric mucosa pentagastrin 10^{-6}M increased HDC activity over basal from 10.46 to 19.72 x 10^{-4} pmol mg tissue^{-1} h^{-1} (n = 4, $p < 0.02$). Pentagastrin 5 x 10^{-6}M in the kitten isolated gastric mucosa also produced a significant elevation of HDC over basal 0.325 to 0.617 x 10^{-4} pmol mg tissue^{-1} h^{-1} (n = 6, $p < 0.02$). Metiamide 10^{-3}M did not reduce the pentagastrin induced elevation of HDC in either the rat or the kitten gastric mucosa.
This study shows that HDC can be reliably measured in rat and kitten mucosa, despite low levels of HDC activity in the latter, using the coupled assay. In the rat and kitten gastric mucosa pentagastrin produced an increase in acid output and also an increase in HDC activity. Metiamide markedly inhibited the acid response to pentagastrin without inhibiting the observed elevation of HDC. This suggests a separation of the acid secretory effect and HDC activation. The discrepancy between the results obtained using the CO_2 trap assay and the coupled assay are difficult to explain but may reflect a lack of specificity in the former assay.

Bunce, K. T. and Parsons, M. E. (1976). J. Physiol. 258, 453 - 465.
Bunce, K. T. et al (1981). Br. J. Pharmac. 72, 561 - 562.
Forte, J. G. et al (1975). J. Physiol. 244, 15 - 31.
Hakanson, R. et al (1974). J. Physiol. 243, 483 - 498.
Keeling, D. J. et al (1982). This meeting.
Kobayashi, Y. and Ivy, A. C. (1959). Am. J. Physiol. 196, 835 - 836.
Lorenz, W. et al (1969). Biochem. Pharmac. 18, 2625 - 2637.
Main, I. H. M. and Pearce, J. B. (1978). J. Pharmac. Methods, 1, 27 - 38.

AXONAL TRANSPORT OF CHOLINE ACETYLTRANSFERASE IN VAGUS AND SCIATIC NERVES IN RATS WITH ACUTE EXPERIMENTAL DIABETES

J. Heidi Mayer and D. R. Tomlinson, Department of Physiology and Pharmacology, Medical School, Queen's Medical Centre, Nottingham NG7 2UH.

The dependence of the axon on delivery of materials from the cell body by axonal transport prompts the suggestion that impairment of this process may contribute to neuropathy in diabetes. Defective axonal transport has been reported in acute experimental diabetes (see review by Sidenius, 1982). This study set out to measure and compare axonal transport in cholinergic neurones of the parasympathetic and motorskeletal systems in rats with short-term experimental diabetes.

Three groups of age-matched male Wistar rats (260-300 g) were established. One group ('Controls') received no treatment. The other two groups were made diabetic by injection of streptozotocin (50 mg/kg i.p.). Either 1 or 3 weeks later tight prolene ligatures were tied around the left vagus (at level C5) and left sciatic (mid-femur level) nerves under pentobarbitone Na anaesthesia with full aseptic technique. The controls were operated upon identically at a time to coincide with the 3 week diabetic group. Twenty four hr later the rats were killed, sciatic and vagus nerves removed, segmented and assayed for choline acetyltransferase (ChAT) as described elsewhere (Tomlinson et al., 1982). Accumulations of ChAT activity proximal and distal to the constrictions were calculated as indices of orthograde and retrograde axonal transport.

At death both groups of diabetic rats had lost weight (1 week - 238 ± 3 (SEM) g; 3 weeks - 229 ± 6 (SEM) g) and were equally hyperglycaemic (fasting blood glucose - 18 to 20 m mol/l). The accumulations are shown in the table. These data show that diabetes initially provoked an increase, followed by a fall, in orthograde transport of ChAT in the sciatic nerve together with a progressive fall in retrograde transport significant at 3 weeks after onset. There were no changes in transport in the vagi.

Accumulations of ChAT activity (n mol ACh/hr/nerve) proximal and distal to 24 h constrictions. Values are means ± SEM; number of rats in brackets.

	L. sciatic nerve		L. vagus nerve	
	Proximal	Distal	Proximal	Distal
Controls (15)	4.3 ± 0.4	0.4 ± 0.2	2.1 ± 0.2	1.0 ± 0.1
1 week diabetes (9)	5.7 ± 0.4 *	-0.6 ± 0.2	2.4 ± 0.4	0.9 ± 0.3
3 weeks diabetes (5)	2.4 ± 0.5 **	-1.2 ± 0.7 *	2.1 ± 0.1	0.8 ± 0.2

By comparison with controls (unpaired t) * denotes $P < 0.05$, ** denotes $p < 0.01$.

We are grateful to the British Diabetic Association for financial support of this work.

Sidenius, P. (1982) Diabetes, 31, 356-363.

Tomlinson, D.R., Holmes, P. R. and Mayer, J.H. (1982) Neuroscience Letters, in press.

POTENTIATION BY LIGNOCAINE OF RESPONSES OF GUINEA-PIG VAS DEFERENS TO SINGLE PULSE FIELD STIMULATION

D.C. Buss, Karen Cheesbrough & T.L.B. Spriggs, Department of Pharmacology and Therapeutics, Welsh National School of Medicine, Heath Park, Cardiff. CF4 4XN

Lignocaine (7.5×10^{-5} to 1.1×10^{-3}) potentiated the responses of the guinea pig vas deferens to single pulse field stimulation in a dose related manner. At greater concentration the potentiation decreased until at 8.8×10^{-3} M responses were impaired or abolished.

Stripped Vasa deferentia from male guinea pigs (300-600 g) were suspended between parallel wire electrodes and superfused or immersed in Krebs Hensleit solution at 37°C and gassed with 5% CO_2 in O_2. Single rectangular pulses of 0.1 msec duration and supramaximal voltage were delivered every 5 min from a digital stimulator/timer (Square One Instruments).

Mepivacaine, an amide which bears some chemical similarity to both lignocaine and prilocaine, produced a dose-related potentiation of responses comparable to that of lignocaine and greater than that of prilocaine. The ester-linked local anaesthetic procaine was devoid of potentiating activity in concentrations up to 10^{-4} M, producing depression of responses at higher concentrations, whereas cocaine had a potentiating effect which was greater than that of prilocaine but less than that of lignocaine or mepivacaine.

Metaraminol, a potent inhibitor of the neuronal uptake-1 process for noradrenaline potentiated responses to single pulse field stimulation to a greater extent that lignocaine. However, the time courses of the tension responses differed, those in the presence of metaraminol being slower to peak tension and of longer duration than those in the presence of lignocaine.

The use of single pulse stimuli at 5 min intervals is considered to militate against released transmitter(s) modulating transmitter release, and hence against offering as an explanation for lignocaine's potentiating action an interference with pre-synaptic receptors.

A SIMPLE MODEL OF A SUCROSE-GAP USED FOR RECORDING MEMBRANE POTENTIAL CHANGES AT THE CHICK NEUROMUSCULAR JUNCTION

F.A. Wali* (introduced by R.C. Elliott), Department of Applied Biology, Brunel University, Uxbridge, Middlesex, UB8 3PH, England.

A simple and small model of a sucrose-gap apparatus used for recording changes in membrane resting potential induced by drug action at the chick biventer cervicis (BVC) nerve-muscle preparation was made from perspex blocks.

The Sucrose-gap apparatus was originally introduced by Stampfli (1954) for the study of myelinated nerve fibres. It has subsequently been used by Bennett & Burnstock (1966) for the study of smooth muscle, and modified by Kosterlitz, Lees & Wallis (1968) and Berger & Barr (1969) for the introduction of rubber membranes on both sides of the sucrose chamber.

In the present model, parafilm was used to isolate the sucrose chamber, and the technique is sensitive to voltage variations as low as 0.01 mV. It is based on bathing a section of the muscle in isotonic sucrose solution, to continuously wash out most of the electrolytes responsible for extracellular potential shunt. It basically consists of 3 chambers: chamber 1 contains the tendon which encloses the motor nerve and is superfused with Krebs solution, chamber 2 contains a section of the muscle nearest to the tendon and is superfused with isotonic sucrose solution, and chamber 3 contains the distal portion of the muscle and is superfused with Krebs solution containing the test drug. All 3 chambers are clamped onto a perspex block and set up in a Faraday cage. Recordings are made by a pair of calomel electrodes in contact with woollen wicks extending from chambers 1 & 3.

Using the present apparatus, the effect of methohexitone (i.v. general anaesthetic) on the depolarizations produced by acetylcholine and tetraethylammonium was studied in the isolated chick biventer cervicis muscle (Wali, 1982).

Bennett, M.R. & Burnstock, G. (1966). J. Physiol. 183, 637-648
Berger, W. & Barr, L. (1969). J. Appl. Physiol. 26, 378-382
Kosterlitz, H.W., Lees, G.M. & Wallis, D.I. (1968). J. Physiol. 195, 39-53
Stampfli, R. (1954). Experientia, 10, 508-509
Wali, F.A. (1982). Br. J. Pharmacol. 76, 186P

* Present address: Anaesthetics Unit, The London Hospital, Medical College, Whitechapel, London E1 1BB.

IS cGMP INVOLVED IN THE SEXUAL DIFFERENTIATION OF THE BRAIN?

Omunnakwe Amechi, Mohsen Ani, Peter J.Butterworth and Patrick J.Thomas, Departments of Pharmacology and Biochemistry, Chelsea College, Manresa Road, London, SW3 6LX.

The sexual differentiation of the rat brain is not directly under genetic control but is brought about by sex hormones during a critical perinatal period. Testicular androgens masculinize the hitherto undifferentiated brain after being converted to oestrogens in the hypothalamic-limbic region; these oestrogens bind to cytoplasmic receptors which move to the nucleus and associate with the chromatin, thereby modifying genetic expression. An early consequence of this is the synthesis of protein(s) which inhibit adenylate cyclase activity. Since cyclic 3'-5'-adenosine monophosphate (cAMP) acts as an organizer in developing neural tissue, we proposed (Ani et al; 1980, Thomas, Ani; 1982) that it is an intermediate through which both neurotransmitters and oestradiol act to confer sexual differentiation upon the brain.

Is cyclic 3'-5'-guansine monophosphate (cGMP) a substance which also has neurotrophic potential, often acting in a manner opposite to that of cAMP (Goldberg et al; 1973, 1975; Greengard, 1978) another such intermediate? The first step towards answering this question is to see whether there are any sexual differences in guanylate cyclase activity in the brains of newborn rats.

We measured enzyme activity in the brain extracts by homogenizing the hypothalamic -amygdaloid regions of 5-day-old Wistar rats in ice cold 0.32M sucrose, centrifuging 1,000 x g for 10 mins to remove cell debris and then at 100,000 x g for 1 hour.

Supernatants and rehomogenized pellets were incubated with (^{3}H)-guanosine-5-triphosphate (GTP) (0.01-0.1mM) in a solution (pH7.5, 37^{o}C) containing the appropriate triphosphate regenerating reagents and co-factors. (^{3}H)cGMP formed was separated by thin layer chromatography and estimated by liquid scintillation counting. After preliminary experiments to determine the initial velocity of reaction and optimal enzyme concentration we found that the Michaelis constants were approximately 0.1mM in both sexes. When simplified assays, using only 100μM substrate were carried out, we obtained the following activities; pellets, male 9.03±1.03, female 6.34±1.02; supernatant, male 18±0.6, female 12±0.6. Data are in nmoles mg^{-} protein $^{-1}$ h^{-1}and for obvious reasons are lower than Vmax values.

In both fractions female values were significantly ($P<0.01$) lower than male. Neonatal castration of males (removal of endogenous androgen) led to values similar to those of females and neonatal administration of masculinizing doses of testosterone propionate (0.05μg daily from birth) to females led to values similar to those of normal males.

Our results suggest that sex steroids affect guanylate cyclase activity in the developing brain in a manner opposite to that on adenylate cyclase and that cGMP may be an intermediate in the sexual differentiation of the brain.

Supported by Wellcome Trust.

Ani, M., Butterworth, P.J. & Thomas, P.J.(1980). Brain Research 183, 341-353.
Goldberg, N.D. (1973). Adv.Cyclic Nucleotide Research 3, 155-223.
Goldberg, N.D. et al (1975). Adv.Cyclic Nucleotide Research 5, 307-330.
Greengard, P. (1978). Science 199, 146-152.
Thomas, P. & Ani, M. (1982). Molecular Aspects of Medicine 5, 1-61.

CALCIUM ANTAGONISTS: DIFFERENTIAL EFFECTS ON GUINEA-PIG AND RAT INTESTINAL SMOOTH MUSCLE

R.F.L. Bates, G.A. Buckley, R.M. Eglen, C.A. McArdle and S.J. Wilkinson, Department of Life Sciences, Trent Polytechnic, Nottingham.

The effects of the calcium slow channel blocking agents (calcium antagonists) on cardiac and vascular smooth muscle have been extensively studied (e.g. Naylor and Poole-Wilson, 1981). There are, however, relatively few reports on the effects of these agents on the smooth muscle of the alimentary tract of species other than the guinea pig. We have compared the effects of the calcium antagonists. verapamil, nifedipine and PY 108-068 upon the response of smooth muscle from the alimentary tract of rats and guinea pigs to stimulation by acetylcholine.

Segments of colon (middle third) and ileum were isolated from rats (Sprague-Dawley, 250g, ♂,♀) or guinea pigs (Duncan-Hartley, 350g, ♂,♂) and suspended under 1g tension in Krebs solution at 37°C, gassed with 5% CO_2 in oxygen. The calcium ion concentration of the Krebs solution was 2.5 $mmol.l^{-1}$. In experiments using lanthanum, the tissue was immersed in Hepes buffered solution containing the same concentration of calcium ion and gassed with oxygen.

Cumululative dose-response curves to acetylcholine were obtained in the presence or absence of the calcium antagonists in normal or calcium deficient Krebs. The antagonists were added 1 or 60 minutes prior to the addition of acetylcholine.

The responses of the guinea pig ileum and colon to acetylcholine were antagonized noncompetitively by all four agents. The responses of both tissues were completely abolished by $10^{-7}M$ of the organic antagonists and $2mmol.l^{-1}$ lanthanum. These results are similar to those of Ticku and Triggle (1976).

In contrast, the rat ileum and colon were relatively insensitive to the organic antagonists which failed to significantly reduce the response to acetylcholine at concentrations up to $10^{-6}M$ (n = 4). Pre-incubation of the rat tissue with the calcium antagonists for periods up to 1 hour did not increase their potency (n = 4) nor was the effect of nifedipine increased by pre-incubation of the tissue in calcium deficient Krebs solution (n = 4), containing the minimum concentration of calcium ions ($0.5mmol.l^{-1}$) required to maintain a maximum response to acetylcholine However, the maximum responses of the rat ileum and colon were reduced (63 ± 3% and 83 ± 7% respectively; $\bar{x}$ ± se, n = 4) by $2mmol.l^{-1}$ lanthanum ion. Pre-incubation of the tissue in calcium free solution abolished the response to acetylcholine.

We conclude that in contrast to similar tissue from the guinea pig, the responses to acetylcholine of the rat ileum and colon are very insensitive to the organic calcium antagonists verapamil, nifedipine and PY 108-068.

The drugs used were generously donated by the following: verapamil (Abbott Laboratories Ltd.), nifedipine (Bayer UK Ltd.) and PY 108-068 (Sandoz Ltd.).

Nayler, W.G. and Ph. Poole-Wilson (1981) Basic Res. Cardiol. 76, 1-15.
Ticku, M.K. and D.J. Triggle (1976) Gen. Pharmacol. 7, 133-140.

STATISTICAL INTERPRETATION OF MICROAUTORADIOGRAPHS USING A LOG-LINEAR MODEL

G.A. Buckley[1], P.B.H. Dixon[2] & A.J. Murphy[3], [1]Department of Life Sciences and [2]Department of Mathematics, Trent Polytechnic, Nottingham, NG1 4BU and [3]Safety Evaluation Group, Fisons plc, Bakewell Road, Loughborough, LE11 0QY

Autoradiography at the cellular level can provide valuable information about the distribution of drugs, but the correct interpretation of the results is dependant on a valid statistical analysis of the grain counts. There have been several methods described for interpretation of electron microscope autoradiographs (Blackett & Parry, 1977, Williams, 1977) but relatively few for use with the light microscope. The most common statistic employed has been chi-squared with the further sophistication of a computer program with a sub-routine to choose factors for minimum chi-squared. We now propose an alternative approach to the problem, in which the results of grain counts are analysed by a model-fitting computer package.

Model fitting is common in many branches of statistics such as regression analysis and analysis of variance. A linear model for the logarithms of frequencies can be developed for contigency table data in which, by analogy with analysis of variance models, main effects and interactions can be identified, with the concept of interaction replacing the more usual idea of association for describing relationships in the qualitative variables under consideration. To assist with the model fitting and the estimation of parameters the GLIM computer package (Baker & Nelder, 1978) can be used interactively.

Using either a photograph or a defined field in the microscope the grains in the autoradiograph are counted and associated with a particular location of the tissue. In the liver parenchyma we used sinusoids, hepatocyte cytoplasm, hepatocyte nucleus, and bile canaliculus. A grid of dots is next superimposed over the same field or photograph and these dots similarly counted and associated with the locations. Grids with 80 and 126 dots at square intervals have been used with exactly similar results. These pseudo grains give an estimate of the area of each location as, a fraction of the total area of field (Chalkley, 1963) and indicate the grain counts to be expected from a uniform distribution of the drug.

The GLIM procedure of fitting models to frequency data is of particular convenience in this case. The modelling process can identify associations between the factors or variables under consideration. Cromoglycate, for example, shows a high concentration in the canaliculi (Buckley et al 1982) and the GLIM program will reveal an improvement in the fitted model if the interaction term "DRUG.LOCATION" is included and will produce a significant parameter estimate for the specific interaction of the drug with the canaliculus location. A further advantage is that other associations can be similarly investigated involving the factors:- field or photograph; animal; time after dosing; drug. Thus the procedure can pick out differences between fields within a liver, differences between animals, changes in distribution of drug with time, and the differences between the distribution of different drugs.

Baker, R.J. & Nelder, J.A. (1978) General Linear Interactive Modelling (GLIM) Release 3. Numerical Algorithms Group, Oxford.

Blackett, N.M. & Parry, D.M. (1977) J. Histochem. Cytochem., 25, 206-214

Buckley, G.A. et al (1982) This meeting

Chalkley, H.W. (1943) J. Natl. Cancer Inst., 4, 47-53

Williams, M.A. (1977) In: Practical Methods in Electron Microscopy Vol. 6. Ed. A.M. Glauert. Elsevier/North Holland, Amsterdam, pp 85-166

DISTRIBUTION OF SODIUM CROMOGLYCATE IN RAT LIVER USING MICROAUTORADIOGRAPHY

G.A. Buckley, A.J. Murphy[1] & M.G. Neale[1], Department of Life Sciences, Trent Polytechnic, Nottingham, NG1 4BU and [1]Safety Evaluation Group, Fisons plc, Bakewell Road, Loughborough, LE11 0QY.

After intravenous administration to the rat sodium cromoglycate is not metabolised and is rapidly eliminated in the bile with 90% appearing in the first hour (Ashton *et al*, 1973). Bile samples were, however, only collected at hourly intervals. Rates of biliary elimination of many other compounds have been studied but usually with first samples no earlier than 10 minutes after administration (e.g. Powell *et al*, 1975). There have been no reports for sodium cromoglycate and very few for other compounds on hepatic distribution at the cellular level using microautoradiographic techniques. We have adapted the early techniques of Appleton (1964) and Stumpf and Roth (1966) to study the hepatic distribution of cromoglycate. This has allowed us to examine critically the disposition of a water-soluble compound at times which may be unrealistic with biliary excretion techniques.

28 Cobs Wistar male rats, (260-300 g), anaesthetised with sodium pentobarbitone, were given an intravenous dose of 11 mg kg^{-1} (^3H) sodium cromoglycate (specific activity 40 $\mu Ci\ mg^{-1}$). The hepatic distribution of radioactivity was studied at the following times after dosing: immediately, 0.5, 1, 1.5, 2, 3, 4, 5, 10 and 30 minutes. Only one liver sample was taken from each rat. One rat received a continuous infusion of 15 mg kg^{-1} over a 15 minute period and was killed immediately the infusion ended. Because of the short time periods involved the abdomen was opened before the dose was administered. At the precise time after dosing a slice of liver was removed and frozen to a chuck using the successive freezing method of Stumpf (1976). 3 µm cryostat sections were cut at -30°C. After freeze-drying, sections were placed in contact with a 3-4 µm layer of Ilford K2 nuclear emulsion on a microscope slide. After 56 days in light-tight boxes at -30°C the slides were photographically developed, fixed and stained with haematoxylin and eosin.

Microscopic examination revealed radioactivity in the bile ducts of the portal tracts as early as 0.5 minutes after administration with a maximum between 2 and 5 minutes. In the parenchyma, radioactivity was concentrated in the bile canaliculi with relatively little in the hepatocytes. Continuous infusion resulted in a similar picture to that at 2 minutes. By 10 minutes after dosing the level of (^3H) cromoglycate in the bile duct was declining and by 30 minutes was detectable at low levels.

The rapid appearance of cromoglycate in the bile raises questions concerning its probable route from blood to bile. The classical route would be across the hepatocyte and, because of the speed involved, this would probably be diffusion of free cromoglycate rather than bound to protein, as suggested for glycocholate (Strange *et al*, 1979). Alternatively cromoglycate may pass directly from the sinusoids to the canaliculi via the intercellular space (Anderson and Javitt, 1974).

Anderson, K.E. and Javitt, N.B. (1974) In: The Liver, Normal and Abnormal Functions Part A, Ed. F.F. Becker, Marcel Dekker Inc. N.Y. 371-400.
Appleton, T.C. (1964) J.Roy.Micr.Soc. 83, 277-281.
Ashton, M.J. *et al* (1973) Toxicol.Appl.Pharmacol. 26, 319-328.
Powell, G.M. *et al* (1975) Biochem.J. 148, 303-307.
Strange, R.C. *et al* (1979) Biochim.Biophys.Acta. 573, 535-545.
Stumpf, W.E. (1976) In: Methods in Cell Biology.Vol.13, Ed. D.M. Prescott, Academic Press N.Y., 171-193.
Stumpf, W.E. and Roth, L.J. (1966) J.Histochem.Cytochem. 14, 274-287.

POSITIVE SCATCHARD PLOTS FOR THE BINDING OF METHYL ORANGE TO BOVINE ALBUMIN: EFFECT OF LIGAND CONCENTRATION

L. Stephen Clegg & W. Edward Lindup, Department of Pharmacology and Therapeutics, University of Liverpool, P.O. Box 147, Liverpool L69 3BX.

The apparent association constant (K) and number of binding sites (n) for some ligands vary inversely with the albumin concentration (Bowmer & Lindup, 1980). Experiments done with a single concentration of such a ligand together with a range of albumin concentrations will produce data which give a positive slope on a Scatchard plot. Cortisol is the only ligand for which the influence of the ligand concentration on the slope of a positive Scatchard plot has been studied (Mueller & Potter, 1981). We have therefore investigated the binding of several concentrations of methyl orange to bovine albumin as a function of albumin concentration. Binding was measured by equilibrium dialysis at 37°C and pH 7.4 (Bowmer & Lindup, 1980).

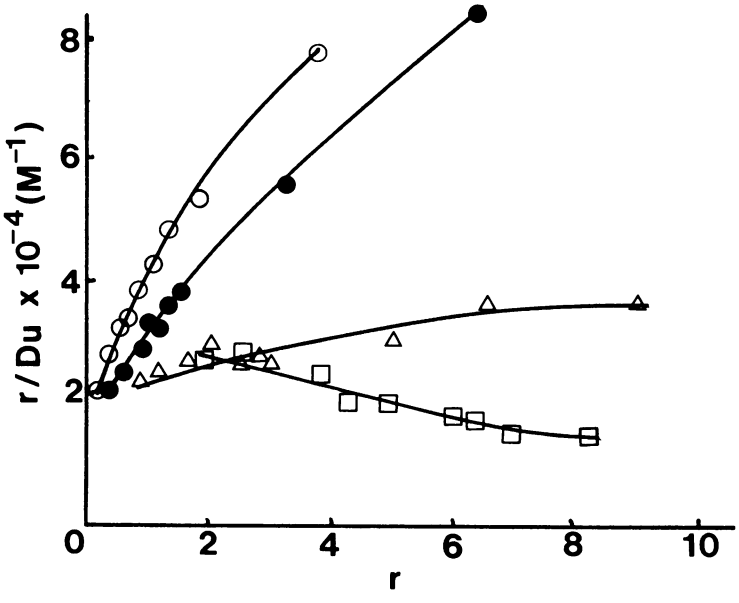


Figure 1. Each curve is a Scatchard plot for the binding of methyl orange (○ 150μM; ● 250μM; △ 500μM; □ 1,500μM) to a range (7.5μM to 600μM) of bovine albumin concentrations.

The binding of a single concentration (150μM) of methyl orange to various concentrations of bovine albumin gave a positive Scatchard plot (Figure 1). Experiments with higher concentrations of methyl orange decreased the slope of the curve until at a concentration of 1,500μM there was a transformation to a negative slope (Figure 1). Similar binding behaviour was observed with porcine albumin (results not shown). Human albumin, which has a higher affinity for methyl orange than either bovine or porcine albumin, did not produce anomalous Scatchard plots (Bowmer & Lindup, 1980). The reason for the species difference in the effect of protein concentration on binding to albumin is not clear but may be connected with differences in affinity.

Bowmer, C.J. & Lindup, W.E. (1980) Biochim. Biophys. Acta, 624, 260-270.
Mueller, U.W. & Potter, J.M. (1981) Biochem. Pharmacol. 30, 727-733.

ERRORS IN K_D ESTIMATES RELATED TO TISSUE CONCENTRATION IN LIGAND BINDING ASSAYS USING FILTRATION

S. Lazareno & S.R. Nahorski, Department of Pharmacology and Therapeutics, Medical Sciences Building, University of Leicester, University Road, Leicester. LE1 7RH.

The dissociation constant (K_D) of a radioligand calculated from saturation experiments should, in theory, be independent of tissue concentration, as long as both free and bound radioligand have been accurately measured or estimated. In preliminary experiments using ^{3}H-domperidone to label dopamine receptors in tissue from both rat striatum and bovine pituitary, the apparent K_D and non-specific binding (NSB) but not the apparent B_{max}, approximately doubled with each four-fold increase in protein concentration. In further experiments, filtration and centrifugation assays were conducted in parallel.

Washed rat striatal homogenates were incubated with ^{3}H-domperidone in Tris-HCl buffer (50 mM, pH 7.6) for 1 hour at 20°C. The tissue was trapped on glass-fibre filters (GF/C), washed with 15 ml buffer, and the bound radioactivity was measured using liquid scintillation counting. Free radioligand was either (a) estimated as the difference between total radioligand in the tube (measured in an aliquot of incubation mixture) and bound radioligand, or (b) measured directly in the supernatant after centrifugation. NSB was defined as binding in the presence of 1 µM d-butaclamol. Different tissue concentrations were obtained by varying the incubation volume (0.25, 1, 4 ml). Use of the supernatant free concentrations in the analysis reduced or eliminated the relationship of apparent K_D with protein concentration (Table 1). The error in the 'estimated' free ligand concentration was caused by a large component of ^{3}H-domperidone binding which was washed off the membranes during filtration. This washable binding, which was completely non-specific and linearly related to protein concentration, varied somewhat between different experiments and tissue preparations (mean = 1046 fmol/mg prot/nM; SD = 306; n = 18).

It can be shown that: (a) K_D'super' = KD'estd'/(1 + (W x P)), and (b) $P_2 = 1/W$; where W = 'washable NSB' (pmol/mg prot/nM), P = protein concentration (mg/ml), and P_2 = protein concentration where K_D'estd' = 2 x K_D'super'. Equation (a) provides a formula for correcting the K_D'estd' with respect to the error introduced by 'washable NSB'. Equation (b) states that the use of estimated - as opposed to directly measured - free ligand concentrations will result in a doubling of apparent K_D at a protein concentration of 1/washable NSB. These relationships, derived from a non-specific binding component, are independent of the B_{max} of the tissue and K_D of the radioligand.

TABLE 1 (n = 3)

Protein (µg/ml)	Free	B_{max} (fmol/mg)	K_D (pM)	NSB (fmol/mg/nM)	Washable (fmol/mg/nM)	Washable (fmol/mg/nM)
1260 ± 56	Estd	347 ± 21	239 ± 22	105 ± 6	987 ± 34	Total binding (n = 6)
	True	352 ± 18	113 ± 6	226 ± 14		1119 ± 107
315 ± 14	Estd	347 ± 14	64 ± 3	213 ± 15	1250 ± 196	
	True	348 ± 14	48 ± 3	292 ± 17		NSB (n = 4)
79 ± 4	Estd	349 ± 20	52 ± 4	342 ± 27		1194 ± 187
	True	354 ± 24	54 ± 1	366 ± 18		

The presence of 'washable NSB' can clearly contribute to errors in estimated K_D related to tissue concentration, though the results in Table 1 indicate that additional factors may be involved (e.g. Lazareno, 1982). 'Washable NSB' can also cause underestimates of the 'bindability' of the radioligand.

Lazareno, S. (1982) J. Receptor Research (in press).

LACK OF CORRELATION BETWEEN OPIATE RECEPTOR BINDING AND BIOLOGICAL PROPERTIES OF MATERIAL EXTRACTED FROM RAT BRAIN

G.D.H. Leach, P.M. Lewis, J.E. Olley and D. Wood, Postgraduate School of Studies in Pharmacology, University of Bradford, Bradford BD7 1DP.

It is generally accepted that exogenous and endogenous opioids which compete with tritiated naloxone (^{3}HNx) for binding sites in brain also inhibit acetylcholine (Ach) release from electrically stimulated guinea pig ileum in a Nx reversible manner (Beddell et al, 1980). This communication reports on a material extracted from rat brain which, although demonstrating affinity for Nx binding sites in brain, produces effects upon the guinea pig ileum which are refractory to Nx.

Brains from four hooded Lister rats were extracted according to the method of Sarne et al (1978) and the residue dissolved in 0.2M acetic acid and applied to a Sephadex G10 column (1.8 x 30 cm) which was eluted in the same solvent at a flow rate of 0.9ml min^{-1}. Eluate equivalent to the void volume (20-25ml) was collected, divided into three equal aliquots and lyophilised. The residue was reconstituted, in 200µl per aliquot, with either TRIS/HCl (pH 7.7 at 0°C) or Krebs' solution.

Receptor binding involved incubation, in triplicate, of 0.25nM ^{3}HNx ± extract (10^{-5} - 10^{-2} dilution) or levorphanol (1nM-10µM) with rat brain homogenate (0.5ml) in the presence of Na^+ (150mM). Incubation of the samples for 2h at 0°C was terminated by rapid filtration. Brain homogenate preparation and radioactivity counting were carried out as previously described (Lewis & Olley, 1980). The nature of the interaction was analysed using Scatchard analysis, which involved the determination of the effect of a constant concentration of extract on the binding of increasing concentrations of ^{3}HNx (0.5 - 16nM).

The effects of the extract and leu-enkephalin (LE) on the isometric responses of the guinea pig ileum (bathed in Krebs' solution at 37°C, gassed with 95% O_2, 5% CO_2) to field stimulation (0.1Hz, 0.3ms, supramaximal voltage) were assessed before and after Nx (1nM - 1µM). The effects of these substances on submaximal contractions to Ach were also examined.

The extracted material inhibited specific ^{3}HNx binding (n=6). Scatchard analysis revealed an increase in the estimated K_D for ^{3}HNx (control 2.43nM, + extract 4.94nM n=2) but no effect on the estimated Bmax (control 14 pmol g^{-1} tissue, + extract 13 pmol g^{-1} tissue) indicating a competitive type of interaction.

Both LE (1nM - 10µM and the extract (10^{-5} - 10^{-2} dilution) produced concentration related inhibition of the response of the ileum to electrical stimulation, the effects being additive, and up to 80% inhibition being achieved with either agonist. No effect was observed on the responses of the ileum to Ach indicating that both agonists presumably inhibited twitch height by reducing transmitter output. Nx antagonised the response to LE but did not affect the response to the extract.

Extraction and characterisation of the material was repeated and a similar qualitative profile of biochemical and biological activity was consistently obtained (n=4).

These results demonstrate that evidence of receptor binding properties cannot be used in isolation as an index of opioid activity and must be substantiated by relevant studies on other biological systems.

Beddell, C.R. et al (1980) Prog. Med. Chem. 17, 1-39
Lewis, P.M. and Olley, J.E. (1980) Clin. Exp. Physiol. Pharmacol. 7, 640
Sarne, Y. et al (1978) Brain Res. 151, 399-403

SIMULATION OF STEADY STATE DRUG CONCENTRATION RESULTING FROM UNINTENTIONAL VARIATION IN DOSE CONTENT

N.A. Orr & D.P. Vaughan (introduced by D.F. Weetman), Department of Pharmaceutics, Faculty of Pharmaceutical Sciences, Sunderland, SR1 3SD.

The concept of a unit dose for medicines is such that a patient can receive a well defined discrete quantity of drug. Although the content uniformity of drug in medicines is often not questioned, there is evidence to suggest that in practice unit doses can vary greatly in the content of active principle. Orr and Sallam (1978) reported that single tablets of ethinyloestradiol varied from 28% to 259% of labelled content. The current situation concerning content uniformity testing is confused with the approach varying according to regulatory authority (Orr, 1981). One failing common to content uniformity tests of all regulatory authorities is that the standard is not directly related to the blood levels of the drug.

Given a probability density function, p.d.f., of the drug content, x, in a batch of tablets, f(x), then the p.d.f. of blood drug concentrations at any time t resulting from a single dose drawn from the original batch is given by

$$\frac{1}{\beta} \cdot f\left(\frac{x}{\beta}\right) \qquad \ldots(1)$$

where β is the blood concentration at time t resulting after a unit dose.

Upon multiple dosing with single doses drawn from the original distribution every τ hours, the resulting p.d.f. at time t, $n\tau < t < (n+1)\tau$, is given by the nth fold convolution function:

$$\prod_{i=o}^{n-1} \left[\frac{1}{\beta_i} \cdot f\left(\frac{x}{\beta_i}\right)\right]^{*} \qquad \ldots(2)$$

where n is the number of doses given and β_i is the blood concentration after a single unit dose at time $(t-i\tau)$ and * represents the convolution operator. To establish if a particular content uniformity distribution produces an acceptable variation in the blood concentration of the drug it is necessary to solve equation 2. The required solution can be achieved by taking Laplace transforms of equation 2 followed by partial fraction expansion and subsequent inversion to the time domain (Vaughan 1982). An analytical solution of 2 has been implemented on a micro computer. This program generates the p.d.f. of blood drug concentrations for a given f(x).

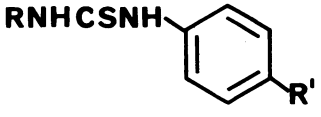
Simulated results demonstrate that the variation in blood concentration depend upon the content uniformity of the doses as well as the kinetics of the drug. We therefore argue that a universal standard for content uniformity cannot be specified and that variations in content uniformity must be controlled on an individual drug basis. This approach requires a definition of the maximum acceptable fluctuation in blood concentrations for a given drug and dosage regimen.

Orr, N.A. & Sallam, E.A. (1978) J.Pharm. Pharmac. 30, 741P.
Orr, N.A. (1981) Progress in the Quality Control of Medicines, Deasy & Timoney (Eds.), Elsevier Biomedical Press.
Vaughan, D.P. (1982) J.Pharm. Sci. in press.

THE SEARCH FOR NEW ANTI-TUBERCULAR AND ANTI-LEPROTIC DRUGS

M. Hooper and S.N. Kulkarni, Department of Pharmaceutical Chemistry, Faculty of Pharmaceutical Sciences, Sunderland Polytechnic, Sunderland SR1 3SD and National Chemical Laboratories, Poona-8, India.

Mono- and disubstituted thioureas are known to exert significant bacteriostatic effects against M. tuberculosis and M. leprae. From the vast number of compounds which have been prepared and tested two have found widespread acceptance, thiambutosine (R = 4 -dimethylaminophenyl; R' = O-n-butyl) and thiocarlide (R = 4-isopentyloxyphenyl; R' = isopentyloxy) (Martindale, 1977). No quantative structure activity relationships (QSAR) have been studied although a number of SAR generalisations have been made.



Series 1 R = 8-ethoxy-4-quinoyl

Series 2 R = 8-n-pentyloxy-4-quinolyl

Series 3 R = 8-chloro-4-quinolyl

For each series R' = H, Me, OMe, OEt, O-n-C_4H_9, O-i-C_4H_9, O-n-C_5H_{11}, Cl, NMe_2.

The novel compounds above were prepared and tested in vitro in India (Kale and Kulkarni, 1979) and the Hansch analysis carried out in Sunderland. A variety of independent variables for the substituent R' in each series were tested namely the lipophilicity constants (π and π^2) (Martin, 1978) and the steric constants, B_1, B_2, B_3, B_4 and L (Verloop et al., 1976). There were extensive cross correlations between the independent variables. The best equation for each series was obtained with the parameter, L, equations 1 - 3. $P < 0.001$.

		n	r	
Series 1	log 1/MIC = 0.44 L + 2.64	9	0.86	1
Series 2	log 1/MIC = 0.48 L + 2.65	9	0.88	2
Series 3	log 1/MIC = 0.40 L + 2.83	9	0.85	3

When the three equations were combined a term in L^2 was just significant at the 95% significance level ($P < 0.05$), equation 4.

	n	r	
log 1/MIC= -0.06 L^2 + 1.04 L + 1.40	27	0.88	4

L_o = 8.67

The correlation coefficient r is very acceptable considering the use of MICs as the biological end point. The caluclated L_o value from equation 4 suggests that the optimum activity is associated with an n-pentyloxy or n-hexyloxy side chain at R'. Thiambutosine (O-n-C_4H_9) and thiocarlide (O-isopentyl) are very close to the optimum structure. This result would predict the lack of activity of mono- and disubstituted aryl thioureas lacking the n-alkyloxy side chain (Kulkarni, 1979). Any developments in this series of compounds should therefore involve variations which retain the essential steric features of this side chain - some proposals will be made.

Kale, K.V. and Kulkarni, S.N. (1977) J. Ind. Chem. Soc. 54, 1104-5.
Kulkarni, S.N. (1979) Ind. J. Chem. Sect. B. 17, 530-2.
Martin, Y. (1978) Quantitative Drug Design Dekker, New York.
Martindale Extra Pharmacoepia 27th Edition (1977) L505 and 1605
Verloop, A. et al., (1976) in Drug Design, E.J. Ariens (ed.) Vol. VII, 165-207.