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Preliminary characterisation of three types of prostanoid receptor mediating smooth muscle contraction

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Comparison of orders of agonist potency is a recognised technique for the pharmacological characterisation of receptors (Furchgott, 1972), and using this approach, we have previously defined some characteristics of a prostanoid receptor mediating smooth muscle relaxation (Apperley, Coleman, Kennedy & Levy, 1979). We have now compared the potencies of five prostanoids in contracting seven isolated smooth muscle preparations to define some characteristics of the receptors mediating these responses. The agonists used were prostaglandins E_1, E_2, $F_{2\alpha}$, the selective leuteolytic $PGF_{2\alpha}$ analogue, ICI 81008 (Dukes, Russell and Walpole, 1974), and the stable PGH_2 analogue, U-46619 (Bundy, 1975).

Guinea-pig ileum, fundus and lung strip, dog and cat iris sphincter muscle, rabbit aorta and dog saphenous vein were suspended in organ baths containing modified Krebs solution with added antagonists (Coleman, Humphrey, Kennedy, Levy & Lumley, 1979) at 37°C or 32°C (ileum only). Agonist concentration-effect curves were constructed cumulatively, and $PGF_{2\alpha}$ was used as a standard in each experiment.

The preparations could be divided into three groups on the basis of orders of agonist potency for producing contraction (Table 1): group I ($E_2 > E_1 > F_{2\alpha} \gg$ U-46619 $\geqslant$ ICI 81008); group II (ICI 81008 = $F_{2\alpha} \gg$ U-46619 = $E_2 > E_1$) and group III (U-46619 $\gg F_{2\alpha} > E_2 > E_1 \geqslant$ ICI 81008. The orders of agonist potency within each group are sufficiently similar to suggest that the receptors involved are similar. On the other hand, the large differences between the orders of agonist potency of the three groups suggest the existence of three pharmacologically distinct receptor types; at the first, PGE_2 is a potent selective agonist, at the second $PGF_{2\alpha}$ and ICI 81008 are potent selective agonists, whilst at the third, U-46619 is a potent selective agonist. Since U-46619 has the same profile of activity as TxA_2 on the tissues used in this study (Coleman *et al.*, 1979), the third type may be a thromboxane receptor. Verification of this hypothesis must await the development of selective antagonists.

Table 1 Comparison of the potencies of PGE_1, PGE_2, $PGF_{2\alpha}$, ICI 81008 and U-46619 for producing contractions of seven isolated smooth muscle preparations

Preparation	*Molar equipotent concentration, $PGF_{2\alpha}$ = 1*				
	PGE_1	*PGE_2*	*ICI 81008*	*U-46619*	*pD_2† of $PGF_{2\alpha}$*
Group I					
Guinea-pig ileum	0.1 (0.06–0.3)	0.02 (0.009–0.05)	⩾110	>30*	6.09 (6.04–6.21)
Guinea-pig fundus	0.2 (0.08–0.3)	0.04 (0.02–0.06)	⩾75	20 (12–33)	6.02 (5.80–6.25)
Group II					
Dog iris sphincter muscle	3000 (790–11300)	515 (179–1483)	0.6 (0.2–1.7)	131 (52–333)	7.96 (7.85–8.08)
Cat iris sphincter muscle	1274 (191–8513)	40 (26–63)	0.4 (0.2–0.8)	63 (29–136)	7.43 (7.28–7.60)
Group III					
Rabbit aorta	29 (15–56)	4.8 (2.3–8.0)	23 (8–65)	0.006 (0.004–0.009)	5.98 (5.89–6.10)
Guinea-pig lung	small relaxation	17 (8–34)	27 (7–116)	0.0009 (0.0004–0.002)	⩽4.5*
Dog saphenous vein	17 (11–27)	4.8 (2.5–9.3)	209 (84–516)	0.003 (0.002–0.007)	6.22 (6.10–6.36)

Each value is the mean of at least 4 observations (95% confidence limits).
† pD_2: negative log of the molar concentration producing 50% maximum response.
* A clearly defined maximum response was not achieved with $PGF_{2\alpha}$ on this preparation; the figure quoted is based on the concentration producing 50% of the maximum response to U-46619, this response was not achieved in all experiments.
≠ Partial agonist, concentration-effect curve not parallel to that of $PGF_{2\alpha}$.

References

APPERLEY, G.H., COLEMAN, R.A., KENNEDY, I. & LEVY, G.P. (1979). The cat isolated trachea, a useful preparation for the study of the smooth muscle relaxant actions of prostaglandins. *Br. J. Pharmac.* **67**, 412–413.

BUNDY, G.L. (1975). The synthesis of prostaglandins endoperoxide analogs. *Tetrahedron Lett.* **24**, 1957–1960.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1979). U-46619, a selective thromboxane A_2-like agonist? *Br. J. Pharmac.* (In Press).

DUKES, M., RUSSELL, W. & WALPOLE, A.L. (1974). Potent luteolytic agents related to prostaglandin $F_{2\alpha}$. *Nature (Lond.).* **250**, 330–331.

FURCHGOTT, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In *Catecholamine, Hand, exp. Pharmac.* N.S. **33**, eds, Blashko, H. & Musholl, E., pp. 283–335, Berlin and Heidelberg, Springer-Verlag.

SC-19220, a selective prostanoid receptor antagonist

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We have suggested the existence of four types of prostanoid receptor. Three of these postulated receptors mediate smooth muscle contraction; the first is characterised by a high sensitivity to PGE_2, the second by a high sensitivity to $PGF_{2\alpha}$ and the third by a high sensitivity to U-46619 (Coleman, Humphrey, Kennedy, Levy & Lumley, this meeting). The fourth receptor mediates smooth muscle relaxation and is characterised by a high sensitivity to PGE_1 and PGE_2 (Apperley, Coleman, Kennedy & Levy, 1979). The hypothesis would be strengthened by the identification of selective antagonists for the postulated

receptors. We now present evidence that SC-19220 (Sanner, 1969) is a specific, selective antagonist at that receptor mediating smooth muscle contraction which shows a high sensitivity to PGE_2.

Guinea-pig ileum, fundus and lung strip, dog and cat iris sphincter muscle, rabbit aorta and dog saphenous vein were suspended in organ baths containing modified Krebs solution with added antagonists (Coleman, Humphrey, Kennedy, Levy & Lumley, 1979). Cat isolated trachea was prepared as described by Apperley *et al.* (1979). Agonist concentration-effect curves were constructed cumulatively, repeated until sensitivity was constant, and then a further concentration effect curve obtained in the presence of SC-19220 (contact time 45 min).

At 3×10^{-4} mol/l SC-19220 had little or no effect on contractile responses of guinea-pig lung, dog saphenous vein and rabbit aorta to PGE_2 or U-46619, dog and cat iris to $PGF_{2\alpha}$ or PGE_2, or on relaxant responses of cat trachea to PGE_1. In contrast, SC-19220 (3×10^{-6}–3×10^{-4} mol/l) caused concentration-related parallel shifts to the right of concentration-effect curves for PGE_2 and $PGF_{2\alpha}$ on guinea-pig ileum and fundus. Analysis of these results (Arunlakshana & Schild, 1959) gave pA_2 values of 5.4 (95% confidence limits 5.3–5.5) and 5.6 (5.4–5.9) on ileum and fundus respectively against PGE_2, slopes of the Schild plots being 1.2 (1.1–1.4) and 1.1 (0.8–1.4) respectively. When $PGF_{2\alpha}$ was the agonist, pA_2 values of 5.3 (5.2–5.5) and 5.2 (5.1–5.4) were obtained on ileum and fundus, with slopes of 0.8 (0.7–0.9) and 0.9 (0.8–1.1). These slopes are sufficiently close to unity to suggest that SC-19220 is a reversible competitive antagonist. The antagonist action of SC-19220 is specific in that, at 3×10^{-4} mol/l, the compound had little or no effect on responses of ileum and fundus to histamine and acetylcholine. Similar results have been reported previously on ileum (Bennett & Posner, 1971).

These results support our previous suggestion (Coleman *et al.*, this meeting) that guinea-pig ileum and fundus contain the same prostanoid receptor and also suggest that PGE_2 and $PGF_{2\alpha}$ act at the same receptor in these preparations. Furthermore, SC-19220 does not antagonise responses mediated by other postulated receptor types. We therefore suggest that SC-19220 specifically blocks the prostanoid receptor characterised by the order of agonist potency $PGE_2 > PGE_1 > PGF_{2\alpha} >> U\text{-}46619 > ICI\ 81008$ (Coleman *et al.*, this meeting).

References

APPERLEY, G.H., COLEMAN, R.A., KENNEDY, I. & LEVY, G.P. (1979). The cat isolated trachea, a useful preparation for the study of the smooth muscle relaxant actions of prostaglandins. *Br. J. Pharmac.* **67**, 412–413.

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac. Chemother.*, **14**, 48–58.

BENNETT, A. & POSNER, J. (1971). Studies on prostaglandin antagonists. *Br. J. Pharmac.*, **42**, 584–594.

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1979). U-46619, a selective thromboxane A_2-like agonist? *Br. J. Pharmac.* (In Press).

COLEMAN, R.A., HUMPHREY, P.P.A., KENNEDY, I., LEVY, G.P. & LUMLEY, P. (1979). Preliminary characterization of three types of prostanoid receptor mediating smooth muscle contraction. *This meeting.*

SANNER, J.H. (1969). Antagonism of prostaglandin E_2 by 1-acetyl-2-(8-chloro-10,11-dihydrobenz[b,f][1,4]oxazepine-10-carbonyl)hydrazine (SC-19220). *Archs int. Pharmacodyn. Ther.*, **180**, 46–56.

Enhanced formation of 6-oxo-$PGF_{1\alpha}$ by ram seminal vesicle microsomes in the presence of anti-oxidants

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Since prostacyclin synthetase can be inhibited by 15-hydroperoxy-arachidonic acid (Gryglewski, Bunting, Moncada, Flower & Vane, 1976), it was suggested that the formation of lipidperoxides could be prevented by anti-oxidants and that these substances could therefore be beneficial in preventing thrombotic complications in atherosclerotic diseases.

In order to study their influence on the formation of 6-oxo-$PGF_{1\alpha}$, ram seminal vesicle microsomes were incubated with increasing concentrations of arachidonic acid (AA) in the presence or absence of various amounts of ascorbic acid and propyl gallate. Indeed, Cottee, Flower, Moncada, Salmon & Vane (1977) reported that ram seminal vesicle microsomes produce only 6-oxo-$PGF_{1\alpha}$ when incubated with small amounts of AA and that an increase of the substrate concentration was accompanied by a decreased formation of 6-oxo-$PGF_{1\alpha}$ concommitantly with an increased production of PGE_2 and $PGF_{2\alpha}$.

Table 1. Formation of 6-oxo-$PGF_{1\alpha}$ by ram seminal vesicle microsomes.
(The results are expressed as a percentage of the amount of 6-oxo-$PGF_{1\alpha}$ formed in the abscence of anti-oxidant; mean ± s.e. mean)

Concentration of AA (*μg/ml*)	*Concentration of ascorbic acid* (*μg/ml*)					
	0	12	24	36	72	100
2	100	108 ± 3.7*	112.4 ± 4.2**	123 ± 10*	130 ± 9**	185 ± 56
5	100	142 ± 12.5**	160 ± 14.2**	154 ± 12***	152.5 ± 28.5	175 ± 39
10	100	145 ± 36	162 ± 14.4**	161.5 ± 15****	165 ± 15***	173 ± 22.5**

Significance: $*P < 0.05$; $**P < 0.025$; $***P < 0.01$; $****P < 0.005$. (Paired t-test)

Enzyme reactions were carried out as described by Cottee *et al.* (1977). Each tube contained lyophilised enzyme preparation (10 mg), 1-^{14}C-AA (250 ng), various concentrations of unlabelled AA and different amounts of anti-oxidants. The reaction products were extracted with ethyl acetate after acidification (pH 3.5), the organic phases were dried, redissolved in chloroform/methanol (2:1, v/v) and developed by TLC (Cottee *et al.*, 1977). The radioactive zones were located by a radiochromatogram scanner, scraped off and counted in a liquid scintillation counter. An increase of the concentration of AA was accompanied by a decreased production of 6-oxo-$PGF_{1\alpha}$. This decrease could partially be prevented by the addition of ascorbic acid in concentrations which did not influence the overall conversion of AA (see Table 1). Similar results were obtained with propyl gallate, 1.25 μg/ml and 2.5 μg/ml ($n = 4$).

From these experiments it can be concluded that the formation of 6-oxo-$PGF_{1\alpha}$ can be enhanced by the anti-oxidants ascorbic acid and propyl gallate. Whether this is due to a stimulation of the synthesis of prostacyclin or by preventing the formation of an inhibitor of the prostacyclin synthetase remains to be elucidated.

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References

COTTEE, F., FLOWER, R.J., MONCADA, S., SALMON, J.A. & VANE, J.R. (1977). Synthesis of 6-keto-$PGF_{1\alpha}$ by ram seminal vesicle microsomes. *Prostaglandins*, **14**, 413–423.

GRYGLEWSKI, R.J., BUNTING, S., MONCADA, S., FLOWER, R.J. & VANE, J.R. (1976). Arterial walls are protected against deposition of platelet thrombi by a substance (prostaglandin X) which they make from prostaglandin endoperoxides. *Prostaglandins*, **12**, 685–713.

Prostacyclin, by preventing platelet activation, prolongs activated clotting time in blood and platelet rich plasma and potentiates the anticoagulant effect of heparin

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At a site of injury platelets adhere, aggregate and develop a procoagulant activity which catalyses the intrinsic blood coagulation pathway. Platelets are thought to accelerate coagulation by changing the structure of the membrane so that certain lipoproteins can act as a platform for clotting (see Marcus, 1978).

Prostacyclin is made by blood vessels and is the most potent inhibitor of platelet aggregation known (Moncada, Gryglewski, Bunting & Vane, 1976). We have now investigated whether prostacyclin influences the role played by platelets in coagulation. Human blood was collected into trisodium citrate and platelet rich plasma (PRP) and platelet poor plasma (PPP) were prepared. Aliquots (200 μl) of blood, PRP or PPP were incubated with either kaolin (20 min) or collagen (3 min) before the addition of calcium chlor-

Table 1. Effect of prostacyclin on kaolin activated clotting time in blood, PRP and PPP with and without heparin.

Sample	Mean clotting time ± s.d. (s) Control	Plus PGI_2 (5 ng/ml)	Significance (paired t-test)
Whole blood	40.0 ± 2.0 $n = 3$	53.5 ± 5.3 $n = 3$	$P < 0.05$
Platelet rich plasma	39.1 ± 3.4 $n = 3$	55.4 ± 3.7 $n = 3$	$P < 0.001$
Platelet poor plasma	95.3 ± 27.5 $n = 3$	94.0 ± 25.0 $n = 3$	NS
Blood + heparin (1 iu/ml)	78.6 ± 6.7 $n = 5$	113.6 ± 13.0 $n = 5$	$P < 0.01$
PRP + heparin (1 iu/ml)	85.8 ± 26.6 $n = 5$	166.0 ± 23.8 $n = 5$	$P < 0.001$
PPP + heparin (1 iu/ml)	312.4 ± 70.9 $n = 5$	286.0 ± 74.6 $n = 5$	NS

ide. The resultant clotting time was measured, (Hardisty & Hutton, 1965). Drugs or vehicle were given 0.5–4 min before kaolin or collagen.

As expected, blood and PRP had shorter clotting times than PPP (Table 1). Incubation with prostacyclin (0.04–10 ng/ml) prior to adding kaolin resulted in significant increases in clotting time in blood and PRP but not in PPP. This effect was dose dependent and occurred in the dose range required to inhibit platelet aggregation. Aspirin (0.1–10 mg/ml), indomethacin (0.1–10 μg/ml) or prostaglandin $F_{2\alpha}$ (0.01–10 μg/ml) did not increase clotting time. Similar results were obtained using collagen.

Heparin prolonged kaolin-clotting time in blood and PRP and even more so in PPP confirming the original observation by Conley, Hartman & Lalley (1948) that heparin activity in plasma is reduced according to platelet concentration. Prostacyclin (0.2–10 ng/ml) potentiated in a dose dependent manner the effects of heparin in blood and PRP but not PPP.

Prostacyclin, therefore, reduces or prevents the development of procoagulant activity associated with platelet activation. The potentiation of the action of heparin is probably also due to inhibition of release of heparin-neutralising activity from platelets.

References

CONLEY, C.L., HARTMAN, R.C. & LALLEY, J.S. (1948). The relationship of heparin activity to platelet concentration. *Proc. Soc. Exp. Biol. Med.*, **69**, 284–287.

HARDISTY, R.M. & HUTTON, R.A. (1965). The kaolin clotting time of platelet-rich plasma: A test of platelet factor 3 availability. *Br. J. Haemat.*, **2**, 258–268.

MARCUS, A.J. (1978). The role of lipids in platelet function: with particular reference to the arachidonic acid pathway. *J. Lipid Res.*, **19**, 793–826.

MONCADA, S., GRYGLEWSKI, R., BUNTING, S. & VANE, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. *Nature*, **263**, 663–665.

Effects of wortmannines, cyclo-oxygenase inhibitors and dexamethasone on enzyme secretion by macrophages

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Secretion of plasminogen activator (P.A.) is characteristic for inflammatory macrophages (Unkeless, Gordon & Reich, 1974), which also secrete, by different mechanisms (Schnyder & Baggiolini, 1978b), lysosomal hydrolases (Schnyder & Baggiolini, 1978a) and lysozyme (Gordon, Todd & Cohn, 1974). Glucocorticosteroids inhibit P.A. secretion and this has been correlated with their anti-inflammatory potency (Vassalli, Hamilton & Reich, 1976).

We report the effects of two classes of anti-inflammatory compounds, wortmannin derivatives (Haefliger & Hauser, 1973; Wiesinger, Gubler, Haefliger & Hauser, 1974) and cyclo-oxygenase inhibitors, on the secretory activities of mouse peritoneal macrophages and we compare them with the effects of dexamethasone. Cells were obtained from thioglycollate-treated OF-1 mice and were cultured under standard conditions (Schnyder & Baggiolini, 1978a). Test compounds (0.1 to 10 μM) were present in the cultures during three days, after which time enzymes were assayed in the media.

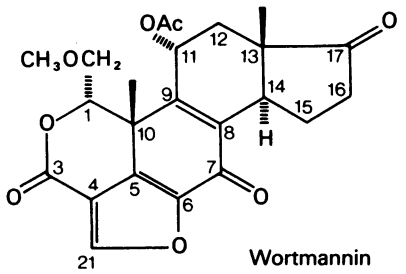

Figure 1 Structure of Wortmannin. The following derivatives were tested: Compound I: 11-desacetoxy, 16β-methyl; compound II: 16-methylene, 17β-hydroxy; compound III: 17α-hydroxy, 17β-vinyl.

Dexamethasone (0.1 or 1.0 μM) does not only block P.A. secretion; it reproducibly lowers the release of lysozyme (by 40% on average) and markedly increases that of lysosomal glycosidases (by 40 to 80%). The latter finding is of importance since the anti-inflammatory action of glucocorticoids has often been related to inhibition of lysosomal enzyme discharge. Wortmannin derivatives, which are structurally similar to steroids (Figure 1) also block P.A. secretion (at 1–10 μM). They have virtually no effect on the lysosomal hydrolases and lower somewhat the release of lysozyme. Like dexamethasone, they do not inhibit cyclo-oxygenase. A striking effect is obtained with cyclo-oxygenase inhibitors. Indomethacin, diclofenac and proquazone (Gubler & Baggiolini, 1978) all markedly enhance P.A. secretion (40 to 100% at 0.1–10 μM) without significantly affecting the release of the other enzymes.

Steroidal and non-steroidal anti-inflammatory compounds have opposite effects on P.A. secretion by macrophages. In this respect, the action of wortmannin derivatives resembles that of glucocorticosteroids.

References

GORDON, S., TODD, J. & COHN, Z.A. (1974). *In vitro* synthesis and secretion of lysozyme by mononuclear phagocytes. *J. Exp. Med.*, **139**, 1228–1248.

GUBLER, H.U. & BAGGIOLINI, M. (1978). Pharmacological properties of proquazone. *Scand. J. Rheumatology, Suppl.*, **21**, 8–11.

HAEFLIGER, W. & HAUSER, D. (1973). Isolierung und Strukturaufklärung von 11-Desacetoxy-wortmannin. *Helv. chim. Acta*, **56**, 2901–2904.

SCHNYDER, J. & BAGGIOLINI, M. (1978a). Secretion of lysosomal hydrolases by stimulated and nonstimulated macrophages. *J. Exp. Med.*, **148**, 435–450.

SCHNYDER, J. & BAGGIOLINI, M. (1978b). Role of phagocytosis in the activation of macrophages. *J. Exp. Med.*, **148**, 1449–1457.

UNKELESS, J.C., GORDON, S. & REICH, E. (1974). Secretion of plasminogen activator by stimulated macrophages. *J. Exp. Med.*, **139**, 834–850.

VASSALLI, J.D., HAMILTON, J. & REICH, E. (1976). Macrophage plasminogen activator: Modulation of enzyme production by anti-inflammatory steroids, mitotic inhibitors, and cyclic nucleotides. *Cell*, **8**, 271–281.

WIESINGER, D., GUBLER, H.U., HAEFLIGER, W. & HAUSER, D. (1974). Anti-inflammatory activity of the new mould metabolite 11-desacetoxy-Wortmannin and of some of its derivatives. *Experientia*, **30**, 135–136.

The effects of indomethacin and BW755C on leukocyte migration and prostaglandin production in carrageenin-induced inflammation

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Cyclo-oxygenase inhibitors such as indomethacin and the salicylates cause a dose-dependent reduction in the concentration of prostaglandins in inflammatory exudates (Higgs, Harvey, Ferreira & Vane, 1976) but the effect of these drugs on leukocyte migration is not so clear. Indomethacin or aspirin do not prevent the generation of 12-L-hydroxyeicosatetraenoic acid (HETE) by arachidonate lipoxygenase (Hamberg & Samuelsson, 1974). HETE is chemotactic for leukocytes (Turner, Tainer & Lynn, 1975) and the failure to inhibit lipoxygenase may explain why the aspirin-like drugs have a relatively weak activity against cell migration. BW755C (3-amino-1-[M-(trifluoromethyl)-phenyl]-2-pyrazoline is equi-active in inhibiting cyclo-oxygenase and lipoxygenase (Higgs, Flower & Vane, 1979) and we have now investigated the effects of indomethacin and BW755C on leukocyte accumulation in carrageenin-induced inflammation.

Polyester sponges, soaked in 2% (w/v) carrageenin in saline were implanted sub-cutaneously in male rats (200 g) and inflammatory exudates were collected after 24 h (Higgs *et al.*, 1976). Total leukocyte

numbers in exudates were estimated on a double blind basis and prostaglandin concentrations were determined by bio-assay for PGE_2-like activity. Each drug was given orally in aqueous vehicle to groups of five rats and similar groups of control animals received vehicle alone. Drugs were given at the time of sponge implantation, 5–8 h later and 3 h before removal of the sponge. Mean prostaglandin concentrations and leukocyte numbers per ml of exudate were calculated and expressed as a percentage of control values in each experiment.

Prostaglandin concentrations in sponge exudates were reduced by indomethacin (ED_{50} = 0.15 mg/kg) and BW755C (ED_{50} = 21.0 mg/kg). Indomethacin (0.5–1.0 mg/kg) caused a significant increase in the numbers of migrating leukocytes whereas higher doses (2–16 mg/kg) reduced leukocyte migration. In groups of rats treated with 0.5 mg/kg indomethacin numbers were 174.9 ± 14.0% of control values (mean ± s.e. mean; n = 5; $P < 0.01$) and at 8 mg/kg cell numbers were 39.0 ± 9.6% of control (n = 5; $P < 0.01$). BW755C (0.2–50 mg/kg) did not increase leukocyte migration at any dose tested but caused a dose dependent reduction in migrating cells. When indomethacin (0.5 mg/kg) was given in combination with BW755C (1–10 mg/kg) there was no increase in leukocyte migration.

These results indicate that indomethacin has a differential effect on leukocyte migration *in vivo*. Potentiation of leukocyte migration occurs at the lowest doses of indomethacin required to abolish cyclo-oxygenase activity. This effect may be explained by a diversion of substrate towards the generation of chemotactic lipoxygenase products. At higher doses, however, cell migration is reduced which may indicate that indomethacin inhibits lipoxygenase at these doses. BW755C is equi-active in reducing prostaglandin concentrations and leukocyte numbers in sponge exudates and this may be due to dual inhibition of arachidonate cyclo-oxygenase and lipoxygenase (Higgs *et al.*, 1979). The theory that the effects of indomethacin and BW755C on leukocyte migration are mediated through lipoxygenase is supported by the observation that BW755C reverses the indomethacin-induced potentiation of migration.

References

HAMBERG, M. & SAMUELSSON, B. (1974). Novel transformations of arachidonic acid in human platelets. *Proc. Natn. Acad. Sci. USA*, **71**, 3400–3404.

HIGGS, G.A., FLOWER, R.J. & VANE, J.R. (1979). A new approach to anti-inflammatory drugs. *Biochem. Pharmacol.*, **28**, 1959–1961.

HIGGS, G.A., HARVEY, E.A., FERREIRA, S.H. & VANE, J.R. (1976). The effects of anti-inflammatory drugs on the production of prostaglandins *in vivo*. In: Advances in prostaglandin and thromboxane research (Eds. B. Samuelsson and R. Paoletti), Vol. 1, p. 105–110. Raven Press, New York.

TURNER, S.R., TAINER, S.A. & LYNN, W.S. (1975). Biogenesis of chemotactic molecules by the arachidonate-lipoxygenase of platelets. *Nature* **257**, 680.

In vivo administration of anti-inflammatory steroids alters activities of enzymes which synthesise and metabolise prostaglandins

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Several recent studies have shown that anti-inflammatory steroids such as prednisolone and dexamethasone reduce the release of prostaglandin-like substances from intact cells (e.g. Lewis & Piper, 1975; Hong & Levine, 1976), most probably by inducing the synthesis of a protein (Flower & Blackwell, 1979) which inhibits the phospholipase-catalysed release of polyunsaturated fatty acid substrates. We show here that these steroids administered *in vivo* have other far-reaching effects on the 'prostaglandin system' by altering the apparent levels of the enzymes concerned with prostaglandin (PG) synthesis and inactivation.

Tissues were removed from male Sprague–Dawley rats pretreated with anti-inflammatory steroids (see below), homogenised in 50 mM phosphate buffer pH 7.4 and 100,000 g fractions prepared for analysis of PG synthesis (resuspended microsomal pellet) and PG inactivation (supernatant). Synthesis was measured by incubating microsomal preparations with arachidonic acid (10 μg/ml) and reduced glutathione (3 mM), followed by extraction and bioassay (Hoult & Moore, 1979). PG inactivation was estimated radiochromatographically utilising $PGF_{2\alpha}$ (10 μg/ml) as substrate (Hoult & Moore, 1977).

Groups of five rats were injected s.c. for eight days with prednisolone (8 mg suspended in 0.9% saline containing carboxymethylcellulose 0.5%, Tween 80 0.4% and ethyl alcohol 0.9%) or with vehicle alone. Prostaglandin synthesis in microsomal fractions pre-

pared from stomach, duodenum and colon was reduced (by 58.6 ± 3.6%, 44.6 ± 6.7% and 26.9 ± 5.9% respectively, $n = 10$, activities compared to those in tissues from control animals), whereas $PGF_{2\alpha}$ breakdown in high-speed supernatants of the same organs was increased (by 26.1 ± 2.9%, 93.7 ± 3.4% and 43.1 ± 8.0% respectively, $n = 10$). In a separate experiment, we found that similar changes in stomach, colon and kidney occurred within two days of treatment (i.e. two injections) at the same dose of prednisolone: PG synthesis depressed by 37.2 ± 1.9%, 68.2 ± 7.1% and 50.9 ± 8.1% respectively, $n = 10$; PG inactivation enhanced by 39.2 ± 2.6%, 91.9 ± 7.1% and 54.2 ± 6.1%, $n = 10$. The effects of three other anti-inflammatory steroids were compared using doses selected to correspond with their established clinical potencies: dexamethasone (1 mg/kg) triamcinolone (5 mg/kg) and hydrocortisone (20 mg/kg). After 2 days treatment, microsomal PG synthesis was reduced in stomach, colon, kidney and lung, whereas the rate of PG inactivation was increased in colon in response to all three drugs and in kidney after triamcinolone treatment. The other organs were not tested for $PGF_{2\alpha}$ inactivation.

These results show that *in vivo* these steroids alter the activity of the prostaglandin system (i.e. the apparent amounts of synthetic and degradative enzymes) in a direction which favours a reduction in PG levels, and which presumably augments their anti-inflammatory effect. However, these experiments do not show whether this is due to alterations in the absolute amounts of the enzymes themselves or of stimulatory/inhibitory factors closely associated with the enzymes. They do show that the anti-prostaglandin actions of these drugs *in vivo* are not limited to direct effects on the phospholipase step in the biosynthetic sequence but have important metabolic effects by altering the activities of enzymes responsible for subsequent steps in the formation and breakdown of these lipids.

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References

FLOWER, R.J. & BLACKWELL, G.J. (1979). Anti-inflammatory steroids induce biosynthesis of a phospholipase A_2 inhibitor which prevents prostaglandin generation. *Nature*, **278**, 456–459.

HONG, S-C.L. & LEVINE, L. (1976). Inhibition of arachidonic acid release from cells as the biochemical action of anti-inflammatory corticosteroids. *Proc. Nat. Acad. Sci. U.S.*, **73**, 1730–1734.

HOULT, J.R.S. & MOORE, P.K. (1977). Pathways of prostaglandin $F_{2\alpha}$ metabolism in mammalian kidneys. *Br. J. Pharmac.*, **61**, 615–626.

HOULT, J.R.S. & MOORE, P.K. (1979). Effects of sulphasalazine and its metabolites on prostaglandin synthesis, inactivation and actions on smooth muscle. *Br. J. Pharmac.*, (in press).

LEWIS, G.P. & PIPER, P.J. (1975). Inhibition of release of prostaglandins as an explanation of some of the actions of anti-inflammatory corticosteroids. *Nature*, **254**, 308–311.

Adaptive changes in activity of prostaglandin synthesising and metabolising enzymes are coupled

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The overall activity of prostaglandins and related substances in a tissue must to a large extent depend upon the balance between their enzymatic synthesis and breakdown; thus the regulation of the relative amounts of these enzymes is a key determinant of the biological functions of prostaglandins (PGs), and is especially relevant in view of the large number of possible physiological and pathological roles assigned to these substances. We have attempted to measure the activities of both PG synthetic and metabolising enzymes in tissues from animals in which different pathophysiological states have been induced so as to find out how adaptations in the prostaglandin system are brought about. The results show that a close reciprocal coupling exists between the amounts of synthetic and degradative enzymes, implying that regulation of prostaglandin activity is under sensitive and perhaps unified metabolic control.

The prostaglandin synthesising and metabolising capacity of the tissues under study were assessed by measuring microsomal conversion of arachidonic acid to bioassayable PGs (synthesis) and inactivation of $PGF_{2\alpha}$ in 100,000 *g* supernatants using the methods cited in the previous communication.

The results are summarised in Table 1. The induction of ulcerous inflammatory lesions in the guinea-pig colon and the rat duodenum (using carrageenan and histamine respectively) was associated with a large increase in the apparent amount of PG synthetase, as measured by microsomal conversion of arachidonate to bioassayable PG-like material. At the

Table 1 Changes in activity of enzymatic prostaglandin synthesis and metabolism in different pathophysiological states.

Species, organ and type of treatment†	*PG synthesis %**		*PG breakdown %**		*Numbers of tests***
Guinea-pig: colon	Exp 1 up	592.0 ± 107.0	down	52.8 ± 2.7	(4,25,8)
(Ulcers induced by oral carrageenan	Exp 2 up	891.0 ± 62.4	down	61.9 ± 7.2	(4,24,8)
administration, 3.0% solution w/v,	Exp 3 up	146.3 ± 25.8	down	58.2 ± 6.0	(5,24,10)
10 days)					
Rat: duodenum	Exp 1 up	201.0 ± 21.2	down	70.6 ± 8.2	(3,8,6)
(Ulcers induced by histamine,	Exp 2 up	264.0 ± 51.1	down	52.4 ± 5.6	(5,10,10)
250 mg i.p., 24 h earlier)					
Rat: kidney	Exp 1 down	76.1 ± 4.3	up	208.4 ± 24.4	(5,8,10)
(Diabetes induced by alloxan					
50 mg/kg i.v., 3 days earlier)					
Rat: stomach	Exp 1 down	58.6 ± 3.6	up	26.1 ± 2.9	(5,10,8)
(Steroid administration, prednisolone					
8 mg/day s.c. for 8 days)					

* Values expressed as per cent change relative to preparations from animals given control treatments.
** (x,y,z): x = no. of animals in group, y = no. of determinations of synthesis, z = no. of determinations of breakdown.
† Full details of these treatments and effects on the prostaglandin system will be presented elsewhere.

same time there was a marked reduction in the apparent content of PG-metabolising enzymes. Both enzyme changes favour enhanced activity of the PG system and a PG excess in these tissues.

By contrast, the two treatments which led to a reduction in apparent PG synthetase content (viz. in the diabetic rat kidney and in the rat stomach following prednisolone treatment) caused enhancement of PG metabolising capacity. Both changes favour diminished activity of the PG system and a PG deficiency.

We conclude that pathophysiological changes in the activity of the PG system are brought about by closely coupled (and mechanistically synchronised?) changes in activity of the enzymes both of PG synthesis and of PG breakdown, at least in the four examples studied, and that both factors are of fundamental importance in evaluating PG turnover and its control. Most likely these results can be accounted for by assuming changes in the absolute amounts of the enzymes themselves, but at present it is not possible to exclude the existence of separate regulatory factor(s) which influence the activity of the synthesising and metabolising enzymes in opposite directions.

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Familiarity-induced feeding in a food-preference test: effects of chlordiazepoxide and naloxone compared with food-deprivation

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When *ad libitum* fed male rats are placed for 10 min in a novel environment, and are given a choice of foods (one of which is the familiar laboratory chow; the other three being palatable but novel), then relatively little eating takes place. With repeated exposure to the same test situation, two reliable behavioural changes occur. Firstly, feeding develops into a major response (familiarity-induced feeding), with latencies to feed becoming short and the total time devoted to feeding becoming prolonged. Secondly, virtually all selection of food comes from the initially novel foods; by the sixth exposure, for example, most animals no longer sample the laboratory chow. This behavioural test system is particularly sensitive to the effects of a single period of imposed food-deprivation (FD).

Following familiarization trials, conducted under non-deprived conditions, 22 h FD exerted two clear effects. Firstly, the total feeding duration was further

enhanced ($t = 8.28$, 34 d.f., $P < 0.0001$), and secondly, the response of consuming the familiar laboratory chow was strongly reinstated, ($t = 5.43$, 34 d.f., $P < 0.0001$). We have proposed (Burton, Cooper & Posadas-Andrews, 1979) that chlordiazepoxide (CDP) given to non-deprived animals mimics the effects of imposed FD. Therefore, CDP administration should produce effects in non-deprived rats that have been familiarized with the food-preference test, which match those of FD. Male rats were injected i.p. 30 min before the test with CDP (5 or 10 mg/kg) or saline ($n = 13$ per group). Chlordiazepoxide (10 mg/kg) did prolong the total eating duration ($t = 5.05$, 24 d.f., $P < 0.0001$) compared with saline controls and reinstated chow feeding most animals ($t = 3.00$, 24 d.f., $P < 0.01$ on log-transformed scores); CDP (5 mg/kg) exerted similar but less marked effects. Hence, CDP can mimic FD in rats familiarized with a food-preference test.

Relatively little progress has been made on the behavioural effects of the opiate antagonist, naloxone, given alone. Only at higher doses is schedule-controlled behaviour in monkeys depressed (Kelleher & Goldberg, 1979). Naloxone (0.3–10 mg/kg) reduces water intake, and at 3–10 mg/kg, reduces food intake in deprived rats (Holtzman, 1979). We ran a preliminary experiment to see whether naloxone could antagonize the effects of imposed FD in animals already familiarized (8 trials) with the food-preference test. Male rats were 22 h food-deprived and injected s.c. 30 min before the test with either naloxone (1.0 mg/kg) or saline ($n = 18$ per group). Naloxone treated animals exhibited a more prolonged total eating duration than saline controls ($t = 2.07$, $P < 0.05$) and spent more time eating the familiar laboratory chow ($t = 2.83$, $P < 0.01$). Hence, naloxone did not counteract the characteristic effects of FD in the test, but actually enhanced them. Further work with naloxone in non-deprived animals will establish whether it can mimic the effects of imposed FD on food-choice.

References

BURTON, M.J., COOPER, S.J. & ROSADAS-ANDREWS, A. (1979). Interactions between chlordiazepoxide and food deprivation determining choice in a food-preference test. *Br. J. Pharmac.* (in press).

HOLTZMAN, S.G. (1979). Suppression of appetitive behavior in the rat by naloxone: lack of effect of prior morphine dependence. *Life Sci.*, **24**, 219–226.

KELLEHER, R.T. & GOLDBERG, S.R. (1979). Effects of naloxone on schedule-controlled behavior in monkeys. In: *Endorphins in Mental Health Research*, Eds. E. Usdin, W.E. Bunney and N.S. Kline, pp. 461–472, Macmillan: London.

Evidence for a role for GABA in benzodiazepine effects on feeding in rats

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Benzodiazepines increase eating in rats, but evidence conflicts as to whether the increase is due to an anxiolytic action, shown by a relative increase in consumption of novel foods (Poschel, 1971), or to a more specific effect on feeding mechanisms, shown by a relative increase in consumption of familiar foods (Cooper & Crummy, 1978).

It has been suggested that benzodiazepines potentiate γ-aminobutyric acid (GABA) transmission (Geller, Taylor & Hoffer, 1978), but little work has been done on possible GABA involvement in the effects of benzodiazepines on feeding behaviour. The experiments described below therefore examined the possible role of GABA in the effects of chlordiazepoxide on feeding behaviour in rats.

The procedure for the first study closely followed Cooper & Crummy (1978). Male hooded Lister rats were food-deprived for 24 h and then allowed 15 min access to a choice of one familiar and four novel foods. There were five groups ($n = 10$). Two were given chlordiazepoxide either 5.0 mg/kg or 10.0 mg/kg (dissolved in saline and injected i.p. in a volume of 1.0 ml/kg), two were given combinations of chlordiazepoxide and the GABA-antagonist picrotoxin (chlordiazepoxide 5.0 mg/kg + picrotoxin 0.50 mg/kg; chlordiazepoxide 5.0 mg/kg + picrotoxin 1.0 mg/kg), and there was a saline control group. There were two identical trials, one week apart.

Chlordiazepoxide alone significantly increased the duration of eating episodes and total eating time in a dose-dependent fashion. The effects were specific to the novel foods, and were antagonized by picrotoxin.

The second study examined the effects of chronic elevation of brain GABA by ethanolamine-O-sulphate (EOS). EOS (5.0 mg/ml) was given in drinking water for one week prior to testing, and its effects on

feeding examined alone, in combination with chlordiazepoxide (2.5 mg/kg), and in combination with picrotoxin (0.50 mg/kg). There was a saline control group, and a group given chlordiazepoxide (2.5 mg/kg) alone. Procedure was identical to the first experiment, except that the choice was between one novel and one familiar food.

EOS alone significantly increased the duration of eating episodes and total eating time, effects specific to novel food. These actions were mildly synergized by chlordiazepoxide, and antagonized by picrotoxin. Chlordiazepoxide significantly increased the time spent eating familiar foods.

This latter effect is opposite to that found with higher doses in the first study, but in line with the findings of Cooper *et al.* (1978). Overall, the results suggest that low doses of chlordiazepoxide increase eating, possibly through a direct action on feeding mechanisms as the increase is specific to familiar foods. Higher doses of chlordiazepoxide and chronic administration of EOS also increase eating, possibly through an anxiolytic action as the increase is specific to novel foods; the anxiolytic action is antagonized, in both instances, by picrotoxin, which suggests that it may be mediated via GABA mechanisms.

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References

COOPER, S.J. & CRUMMY, Y.M.T. (1978). Enhanced choice of familiar food in a food preference test after chlordiazepoxide administration. *Psychopharmacology*, **59**, 51–56.

GELLER, H.M., TAYLOR, D.A. & HOFFER, B.J. (1978). Benzodiazepines and central inhibitory mechanisms. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **304**, 81–88.

POSCHEL, B.P.H. (1971). A simple and specific screen for benzodiazepine-like drugs. *Psychopharmacologia* (Berl.), **19**, 193–198.

Stereotypy of a learned response after apomorphine

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Behaviour affected by many drugs, including amphetamine and apomorphine, is often described as 'stereotyped'. Stereotypy can be defined as the repetitive performance of invariant sequences of behaviour without apparent purpose, and has been found in all mammalian species studied, including man (Randrup & Munkvad, 1967). Measurement of stereotypy has generally employed rating scales which assess subjectively the form and repetitiveness of observed behaviour (e.g. Creese & Iversen, 1973). Such scales have several disadvantages: (i) ratings are subjective; (ii) scales constitute only an ordinal continuum of measurement, and frequently the assumption of even an ordinal scale is contradicted (e.g. Sahakian & Robbins, 1975), and (iii) scales generally confound intensity of stereotypy with the type of response observed.

The present experiments used an arbitrary, learned response and measured automatically its stereotyped nature after treatment with the dopamine agonist apomorphine. Effects on stereotypy of the dopamine antagonists clozapine and α-flupenthixol were also investigated.

Five male hooded rats were food-deprived and trained to respond in an operant chamber containing two levers and a food-pellet dispenser. Food was presented contingent upon lever-pressing according to the following schedule. On each trial, one of the two levers was randomly selected ($P = 0.5$) to provide a food-pellet following a lever-press. Pressing the 'unselected' lever had no effect. When a pellet was delivered, responses on either lever had no effect until the pellet was collected, upon which the next trial would begin. Performance stabilized after about 15, 30 min sessions and was characterized by approximately equal rates of responding on both levers. The effect of apomorphine hydrochloride (0.01–3.0 mg/kg, s.c.) was then determined in duplicate mixed sequences of doses, with each dose preceded by a control session when drug vehicle was administered.

Apomorphine produced a dose-dependent reduction in the number of food-pellets earned ($P < 0.001$). This reduction was accompanied by changes in the pattern of lever-pressing such that there were both more lever-presses after food delivery and more repetitions of ineffective responses on the lever 'unselected' for food delivery. These effects produced reductions in efficiency of performance. Efficiency was measured as the $\log_{10}$ ratio of total lever-presses to total food-pellets earned $+1$. Increasing values for this ratio reflect decreasing efficiency. Apomorphine produced significant dose-dependent increases in this ratio from 0.23 ± 0.02 (control) to 2.05 ± 0.15 (3.0 mg/kg) (mean $\pm$ s.e. mean). Lever-pressing under apomor-

phine fulfilled two criteria for stereotypy, being both repetitive and without apparent purpose. This behaviour differs in form from the gnawing, licking, head-movements and locomotion, seen in untrained rats treated with apomorphine (Ljungberg & Ungerstedt, 1977).

Clozapine (0.3–10 mg/kg) did not affect the response to apomorphine (3.0 mg/kg), even at doses that abolished normal performance. α-Flupenthixol (0.1–1.0 mg/kg) in contrast, improved efficiency at 1.0 mg/kg. This improvement was produced by reduction in lever-pressing rather than by increases in pellets earned. α-Flupenthixol therefore, while antagonizing a stereotyped response, did not reinstate normal performance.

These experiments demonstrate that an arbitrary learned response can become stereotyped after apomorphine, and show that antagonism of stereotypy by neuroleptics may not be accompanied by an objectively-defined normal performance.

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References

CREESE, I. & IVERSEN, S.D. (1973). Blockage of amphetamine induced motor stimulation and stereotypy in the adult rat following neonatal treatment with 6-hydroxydopamine. *Brain Res.*, **55**, 369–382.

LJUNGBERG, T. & UNGERSTEDT, U. (1977). Different behavioural patterns induced by apomorphine: Evidence that the method of administration determines the behavioural response to the drug. *Eur. J. Pharmac.*, **46**, 41–50.

RANDRUP, A. & MUNKVAD, I. (1967). Stereotyped activities produced by amphetamine in several animal species and man. *Psychopharmacologia (Berl.)*, **11**, 300–310.

SAHAKIAN, B.J. & ROBBINS, T.W. (1975). Potentiation of locomotor activity and modification of stereotypy by starvation in apomorphine-treated rats. *Neuropharmacology*, **14**, 251–257.

The pharmacology of the behavioural syndrome induced by systemic kainic acid administration in the rat

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Kainic acid (KA) is a powerful analogue of glutamate (Glu) with marked neurotoxic effects and has been widely used as a tool to induce selective brain lesions after local injections (McGeer, McGeer & Hattori, 1978). However, KA also induces behavioural effects after systemic injections. Thus, KA induces convulsions, 'wet-dog shakes' (WDS) and stereotypies in rats after intraperitoneal (i.p.) or subcutaneous (s.c.) injections (Lanthorn & Isaacson, 1978; Fuller & Olney, 1979; Lenicque, Wepierre & Cohen, 1979). The present report extends the observations on the behavioural effects of s.c. injections of KA in rats.

Male Sprague–Dawley rats (CD COBS, Charles River, France) weighing 180 to 220 g were used. Animals were observed for behaviour during 3 h after KA injections (2.5 to 10 mg/kg, s.c.). For pharmacological interactions, drugs studied were injected i.p. at different time intervals before KA (7.5 or 8.75 mg/kg, s.c.) and the rats were observed at 60 and 90 min (number of WDS in 2 min) or at 150 min (convulsions) after KA injection.

KA induced a typical WDS behaviour, at doses ranging from 5 to 10 mg/kg, with the higher doses being toxic. The behaviour commenced at 30 min and lasted for 2 h with a maximum WDS count at 60–90 minutes. However, after 150 min, WDS disappeared and were replaced by marked clonic convulsions, appearing in 55% animals after KA (8.75 mg/kg).

This WDS behaviour (KA, 8.75 mg/kg, s.c.) was dose-dependently antagonized by mianserin, haloperidol and amino-oxyacetic acid (AOAA) with ED_{50}s of 1.3, 0.125 and 11 mg/kg, i.p., respectively. Cyproheptadine also antagonized WDS at 0.15 and 0.3 mg/kg i.p. (50% antagonism), but this action disappeared at higher doses (0.6 and 1 mg/kg, i.p.). Conversively, KA-induced WDS (KA, 7.5 mg/kg, s.c.) were potentiated by imipramine (0.3 mg/kg, i.p.), tranylcypromine (0.3 mg/kg, i.p.), fluoxetine (1 and 3 mg/kg, i.p.) and bicuculline (0.01 and 0.03 mg/kg, i.p.); however, all those drugs exhibited a bell-shaped dose response curve, with higher doses being inactive. This may be due to a concomitant increase of the toxic effects of KA. In contrast to the WDS, KA-induced convulsions (8.75 mg/kg, s.c., 150 min) were antagonized only by AOAA and diazepam, but not by cyproheptadine, mianserin or haloperidol.

These results suggest that the KA-induced WDS may be, in part, mediated via an increase in central serotonin mechanisms. Thus, this behaviour is quite similar to that induced by 5-HTP (Bédard & Pycock, 1977), which is similarly blocked by cyproheptadine,

mianserin and haloperidol (Bédard & Pycock, 1977; Michaluk & Antkiewicz-Michaluk, 1978) or AOAA, and potentiated by fluoxetine and tranylcypromine (in mice: Worms, unpublished data).

References

BÉDARD, P. & PYCOCK, C.J. (1977). Wet-dog shake behaviour in the rat: a possible quantitative model of central 5-hydroxytryptamine activity. *Neuropharmacology*, **16**, 663–670.

FULLER, T.A. & OLNEY, J.W. (1979). Effects of morphine or naloxone on kainic acid neurotoxicity. *Life Sci.*, **24**, 1793–1798.

LANTHORN, T. & ISAACSON, R.L. (1978). Studies of kainate-induced wet-dog shakes in the rat. *Life Sci.*, **22**, 171–178.

LENICQUE, P.M., WEPIERRE, J. & COHEN, Y. (1979). Protection by 4-amino-hex-5-enoic acid (γ-vinyl GABA) and hypobaric hypoxia against kainic acid neurotoxicity. *Psychopharmacology*, **66**, 51–53.

MCGEER, P.L., MCGEER, E.G. & HATTORI, T. (1978). Kainic acid as a tool in neurobiology. In: *Kainic Acid as a Tool in Neurobiology*, eds. McGeer, E.H., Olney, J.W. and McGeer, P.L., pp. 123–138, Raven Press, New York.

MICHALUK, J. & ANTIKIEWICZ-MICHALUK, L. (1978). The effect of neuroleptics on serotonergic system of the rat brain. *Pol. J. Pharmacol. Pharm.*, **30**, 233–243.

Striatal dopamine receptor activity during 6 months withdrawal following one years neuroleptic administration to rats

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Administration of neuroleptic drugs to male Wistar rats for up to one year produces an enhancement of striatal dopamine receptor activity while drug intake continues (Clow, Jenner, Theodorou & Marsden, 1979; Clow, Jenner & Marsden, 1979). We now report changes in striatal dopamine receptor activity occurring during a six month withdrawal period in these rats following administration of trifluoperazine hydrochloride (2.5–3.5 mg kg^{-1} day^{-1}; TFP) or thioridazine hydrochloride (30–40 mg kg^{-1} day^{-1}; TD2) for 12 months, in comparison to age-matched control animals.

Administration of TFP or TD2 for 12 months induced a greater incidence of spontaneous mouthing movements than seen in the control animals (Number of movements during a 3 min observation period: control animals 16.2 ± 2.0; TFP group 51.2 ± 4.8, $P < 0.05$; TD2 group 55.3 ± 7.5, $P < 0.05$). The increase in mouthing movements disappeared following drug withdrawal such that at two weeks and thereafter no difference was observed from the age-matched control group (number of mouthing movements two weeks following withdrawal: control animals 5.2 ± 1.1; TFP group 5.4 ± 1.2, $P > 0.05$; TD2 group 7.6 ± 1.0, $P > 0.05$).

The enhanced stereotyped response to apomorphine (0.5 mg/kg s.c. 15 min previously) produced during TFP or TD2 administration (control group score 2.5 ± 0.2; TFP group 4.0 ± 0, $P < 0.05$; TD2 group 3.7 ± 0.2, $P < 0.05$) was maintained for 2 weeks following TD2 withdrawal (control group score 2.1 ± 0.1; TD2 group 3.1 ± 0.2; $P < 0.05$) and for one month following TFP withdrawal (control group score 2.0 ± 0; TFP group 2.8 ± 0.2, $P < 0.05$) but thereafter was not different from that observed in the age-matched control group.

The increase in specific [^{3}H]-spiperone (0.125–4.0 nM) striatal binding sites (Bmax) (as judged using dopamine 10^{-4} M) produced by administration of TFP or TD2 for 12 months (binding sites as % control group: TFP group 164 ± 6%, $P < 0.05$; TD2 group 162 ± 13%, $P < 0.05$) was maintained for up to three months following drug withdrawal (binding sites as % control group: TFP group 160 ± 10%, $P < 0.05$; TD2 group 146 ± 11%, $P < 0.05$). Thereafter, while [^{3}H]-spiperone binding sites in TFP treated animals returned to control values by six months, the number of binding sites in tissue from TD2 treated animals were less than those in tissue from age-matched control animals (binding sites as % control group: TFP group 86 ± 12%, $P > 0.05$; TD2 group 80 ± 10%, $P < 0.05$).

The dissociation constant (K_D) for specific [^{3}H]-spiperone binding was elevated by TFP or TD2 administration for 12 months (K_D as % control values: TFP group 539 ± 29%, $P < 0.05$; TD2 group 247 ± 36%, $P < 0.05$) but fell to control values two weeks following withdrawal (K_D as % control values: TFP group 73 ± 10%, $P > 0.05$; TD2 group 72 ± 15%, $P > 0.05$) and remained unchanged thereafter.

The enhanced stimulation of striatal dopamine (1–150 μM) sensitive adenylate cyclase caused by TFP or TD2 administration persisted for the six month withdrawal period (stimulation above basal levels with 50 μM dopamine as % of control values: 12

months TFP group 186 ± 18%; 12 months TD2 group 149 ± 15%; 6 months withdrawal from TFP 161 ± 8%; six month withdrawal from TD2 177 ± 12%, $P < 0.05$).

Withdrawal of animals from 12 months continuous TFP or TD2 administration caused no further increase in striatal dopamine receptor supersensitivity. The duration of the enhanced response varied with each parameter measured. However, the increased sensitivity of striatal adenylate cyclase persisted for at least six months following withdrawal when all other indices of supersensitivity had returned to control levels.

References

CLOW, A., JENNER, P., THEODOROU, A. & MARSDEN, C.D. (1979). Striatal dopamine receptors become supersensitive while rats are given trifluoperazine for six months. *Nature*, **278**, 59–61.

CLOW, A., JENNER, P. & MARSDEN, C.D. (1979). Changes in dopamine mediated behaviour during one year's neuroleptic administration. *Eur. J. Pharmac.* (In press).

Dopamine-sensitive adenylate cyclase in carp retinal homogenates

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It is now well established that the presence of dopamine in various areas of the central nervous system is synonymous with the occurrence of a dopamine-sensitive adenylate cyclase (Iversen, 1975). In the retina of teleost fishes, such as carp and goldfish, dopamine is confined to interplexiform cells, which extend processes in both plexiform layers and make synaptic connections with horizontal, bipolar and amacrine cells (Dowling & Ehinger, 1978). We describe here the presence of a dopamine-sensitive adenylate cyclase in homogenates of carp retina.

Adenylate cyclase activity was estimated using the method of Kebabian, Petzold & Greengard (1972) and the resulting cyclic AMP was determined according to Brown, Ekins & Albano (1972). Mean basal cyclic AMP production was 6.1 ± 0.51 pmol cyclic AMP/mg protein/min ($n = 36$), rising to 41.3 ± 3.3 pmol cyclic AMP/mg protein/min ($n = 36$) in the presence of a maximally stimulating concentration of dopamine (100 μM). This approximate 7-fold stimulation represents a far larger response than hitherto observed in homogenates of other brain regions.

In carp retinal homogenates the EC50 value for dopamine (concentration producing 50% of maximum response) is approx. 1 μM, which is about 10-times less than the EC_{50} values for (−)-noradrenaline and (−)-adrenaline. However the maximum cyclic AMP response evoked by higher concentrations of these agents is similar to that obtained with dopamine. In contrast, histamine, 5-hydroxytryptamine and the classic α- and β-adrenoceptor agonists, (−)-phenylephrine and (±)-isoprenaline respectively, were all inactive.

Dopamine-stimulated adenylate cyclase activity in carp retinal homogenates is potently antagonised by various neuroleptics, whereas α- and β-adrenoreceptor blocking agents are essentially inactive (Table 1). The calculated inhibition constants (Ki) for these drugs in the carp retina are very similar to those previously obtained in homogenates of rat striatum.

Table 1 Comparison of the inhibition constants (Ki) of various neuroleptic and antagonist drugs for dopamine-sensitive adenylate cyclase in homogenates of carp retina and rat striatum

	Ki (M)	
Drug	*Carp retina*	*Rat striatum**
Fluphenazine	3.0×10^{-9}	4.3×10^{-9}
α-Flupenthixol	5.1×10^{-9}	1.0×10^{-9}
(+)-Butaclamol	2.5×10^{-8}	8.8×10^{-9}
Chlorpromazine	7.3×10^{-8}	4.8×10^{-8}
Thioridazine	8.4×10^{-8}	1.3×10^{-7}
Pimozide	1.1×10^{-7}	1.4×10^{-7}
Clozapine	1.7×10^{-7}	1.7×10^{-7}
Spiperone	6.1×10^{-7}	9.5×10^{-8}
Phentolamine	$>1 \times 10^{-6}$	$>1 \times 10^{-6}$
Propranolol	$>1 \times 10^{-6}$	$>1 \times 10^{-6}$
(−)-Butaclamol	$>1 \times 10^{-6}$	$>1 \times 10^{-6}$

Ki values were calculated from the relationship;

$$Ki = \frac{IC_{50}}{1 + S/Km}$$

where IC_{50} is the concentration of drug required to produce 50% inhibition of the maximum dopamine-induced response, S is the concentration of dopamine used (100 μM) and Km is the concentration of dopamine producing half maximal stimulation of adenylate cyclase (1 μM). Six concentrations of each drug (0.01–100 μM) were examined on dopamine-stimulated adenylate cyclase activity (100 μM) on 2–4 separate occasions involving quadruplicate determinations.

* Taken from Iversen (1975).

These data suggest that the carp retina contains specific dopamine-sensitive adenylate cyclase, similar pharmacologically to that previously reported in mammalian systems.

References

BROWN, B.L., EKINS, R.P. & ALBANO, J.M.D. (1972). Saturation assay for cyclic AMP using endogenous binding protein. In: *Advances in Cyclic Nucleotide Research*, eds. Greengard, P. & Robison, G.A., pp. 25–40, Raven Press, New York.

DOWLING, J.E. & EHINGER, B. (1978). The interplexiform cell system. I. Synapses of the dopaminergic neurones in the goldfish retina. *Proc. R. Soc. Lond. B.*, **201**, 7–26.

IVERSEN, L.L. (1975). Dopamine receptors in the brain. *Science*, **188**, 1084–1089.

KEBABIAN, J.W., PETZOLD, G.L. & GREENGARD, P. (1972). Dopamine-sensitive adenylate cyclase in the caudate nucleus of rat brain and its similarity to the 'dopamine receptor'. *Proc. natn. Acad. Sci. U.S.A.*, **69**, 2145–2149.

Time course of degeneration of bulbo-spinal 5-HT/SP neurones after 5,7-dihydroxytryptamine

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Considerable evidence now exists to suggest that the projections of certain medullary raphe neurones to the spinal cord contain both substance P (SP) and 5-hydroxytryptamine (5-HT) (Chan-Palay, Jonsson & Palay, 1978; Hökfelt, Ljungdahl, Steinbusch, Verhofstad, Nilsson, Brodin, Pernow & Goldstein, 1978; Björklund, Emson, Gilbert & Skagerberg, 1979). Like other 5-HT containing neurones, these 5-HT/SP neurones are damaged by 5,6- and 5,7-dihydroxytryptamine (5,6- and 5,7-DHT) (Björklund *et al.*, 1979). Following such lesions, we have studied the time course of the loss of SP and of 5-HT uptake sites from rat spinal cord.

Rats were pretreated with desmethylimipramine (25 mg/kg). One hour later each animal received an injection into the lateral ventricle of 200 μg of 5,7-DHT (freebase) in 20 μl of saline containing ascorbic acid (0.2 mg/ml). The SP contents of ventral spinal cord samples from lesioned animals were determined by radioimmunoassay (Kanazawa & Jessell, 1976). Ventral cord was used since this region contains only SP in descending nerve fibres. High affinity uptake of [^{3}H]-5-HT (10^{-7} M) was measured in homogenates of whole spinal cord from the same animals (Horn, 1973).

One day after the injection of 5,7 DHT, SP and 5-HT uptake were significantly reduced in the cervical, but not in the lumbar spinal cord (Table 1). The SP content and the number of 5-HT uptake sites in lumbar spinal cord were not reduced until day 3. At 5 days, there was almost total loss (80–90%) of ventral SP and of 5-HT uptake sites in both regions of the cord.

These results show the rate of loss of SP from the two regions of rat spinal cord, to correlate well with the loss of 5-HT uptake sites following lesioning with 5,7 DHT. This is consistent with the possibility that SP and 5-HT are stored together in the terminals of nerve fibres originating in the lower medulla. The more rapid neuronal degeneration in the cervical cord may be due to its exposure to a higher concentration of 5,7 DHT than the lumbar cord.

R.F.T.G. is an MRC Student.

Table 1 SP content and [^{3}H]-5-HT uptake in spinal cord after 5,7 DHT.

	Control SP content in ventral cord (pg/mg tissue)	*% control SP content after 5,7 DHT*		*Content [^{3}H]-5-HT uptake (pmol/5 min/mg protein)*	*% control [^{3}H]-5-HT uptake after 5,7 DHT*	
		1 day	*3 days*		*1 day*	*3 days*
Cervical spinal cord	26 ± 2 (9)	32 ± 15* (3)	21 ± 6* (3)	1.7 ± 1 (8)	27 ± 13* (3)	15 ± 9* (3)
Lumbar spinal cord	49 ± 6 (9)	87 ± 5 (3)	34 ± 6* (3)	2.0 ± 0.2 (8)	87 ± 5 (3)	37 ± 6* (3)

Values are expressed as means ± s.e. mean. Numbers in parentheses indicate number of animals.
* denotes $P < 0.05$ by Students *t*-test.

References

BJÖRKLUND, A.J., EMSON, P.C., GILBERT, R.F.T. & SKAGERBERG, G. (1979). Further evidence for the possible coexistence of 5-hydroxytryptamine and substance P in medullary raphe neurones of rat brain. *Br. J. Pharmac.*, **66**, 112P–113P.

CHAN-PALAY, V., JONSSON, G. & PALAY, S.L. (1978). On the coexistence of serotonin and substance P in neurons of the rat's central nervous system. *Proc. Natl. Acad. Acad. Sci. USA*, **75**, 1582–1586.

HÖKFELT, T., LJUNGDAHL, A., STEINBUSCH, H., VERHOFSTAD, A., NILSSON, G., BRODIN, E., PERNOW, B. & GOLDSTEIN, M. (1978). Immunohistochemical evidence of substance P-like immunoreactivity in some 5-hydroxytryptamine-containing neurons in the rat central nervous system. *Neurosci.*, **3**, 517–538.

HORN, A.S. (1973). Structure activity relations for the inhibition of 5-HT uptake into rat hypothalamic homogenates by serotonin and tryptamine analogues. *J. Neurochem.*, **21**, 883–888.

KANAZAWA, I. & JESSELL, T.M. (1976). Post-mortem changes and regional distribution of substance P in the rat and mouse nervous system. *Brain Res.*, **117**, 362–367.

Olfactory bulbectomy induced deficit in passive avoidance acquisition: restoration by muscimol, SL 76 002, fenfluramine, quipazine and fluoxetine

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The olfactory bulbectomy induced deficit in the acquisition of a passive avoidance task can be restored by a number of antidepressant drugs. As neuroleptics, benzodiazepines and psychomotor stimulants are inactive in this respect, the bulbectomy induced learning deficit is proposed to be useful for testing compounds for putative antidepressant properties (Rigter, Van Riezen & Wren, 1977; Cairncross, Cox, Forster & Wren, 1978). To evaluate this model further, we have treated bulbectomized rats with an α-noradrenergic stimulant (clonidine), GABA-mimetic drugs (muscimol and SL 76 002; Lloyd, Worms, Depoortere & Bartholini, 1979), and also drugs which enhance serotonin mediated events (fenfluramine, quipazine or fluoxetine; Wong, Horng, Bymaster, Hause & Molloy, 1974).

Rats (180–200 g, CD- COBS, Charles River, France) were bulbectomized under pentobarbital anaesthesia (50 mg/kg) as described by Cairncross *et al.* (1978). On the seventh post operative day, drugs were administered and the animals were tested for their ability to acquire a passive avoidance task. At each trial in this task the rat was placed on a safe platform above a grid floor and was punished with foot-shock if it stepped off within 3 minutes.

Table 1 indicates the number of trials necessary to acquire the passive avoidance task under different drug treatment. Clonidine was ineffective in restoring the passive-avoidance deficit. The effectiveness of the GABA-like drugs SL 76 002 and muscimol was unexpected and supports the antidepressant potential of SL 76 002 in man (Morselli, Bossi, Henry, Zarifian & Bartholini, 1979). That these drugs are working by a GABA-mediated event is confirmed by the antagonism by bicuculline. The serotonin-potentiating drugs fluoxetine, quipazine and fenfluramine were effective in improving the passive avoidance acquisition of the lesioned rats. Methergoline antagonized the effect of quipazine and fenfluramine, demonstrating that these drugs likely act via serotonergic mechanisms. However, the effectiveness of fenfluramine does not support the bulbectomy model as a reliable predictor for antidepressant activity as this drug has no anti-depressant effect to our knowledge. As serotonin-like compounds are shown here to be effective in the bulbectomy model one wonders whether the effectiveness of a number of anti-depressant drugs in this model is not based on their property to block serotonin-uptake. In support of this we found that the effect of a 5 day imipramine treatment in the model can be antagonized by methergoline pretreatment.

Table 1

Group		*Dose (mg/kg)*	*Route*	*Number of Animals*	*Trials to criterion (mean ± s.e. mean)*	*Statistical significance t-test*
Control sham operated				9	2.8 ± 0.4	
Bulbectomy 7th day				51	6.6 ± 0.6	<0.001[a]
Bulbectomy 11th day				8	6.3 ± 0.9	<0.001[a]
Bulbectomy 7th day	+ fenfluramine HCl	3	i.p.	7	2.9 ± 0.3	<0.01[b]
	+ quipazine	5	i.p.	16	4.0 ± 0.3	<0.01[b]
	+ fluoxetine	10	i.p.	8	3.4 ± 0.4	<0.01[b]
	+ imipramine 5d	10	i.p.	15	4.5 ± 0.5	<0.02[b]
	+ clonidine	0.1	i.p.	8	6.9 ± 0.8	NS
	+ clonidine	0.5	i.p.	8	5.2 ± 0.7	NS
	+ muscimol	1	i.p.	14	4.2 ± 0.6	<0.02[b]
	+ SL 76 002	50	i.p.	8	5.6 ± 0.5	NS
	+ SL 76 002	100	i.p.	7	4.1 ± 0.3	<0.01[b]
	+ SL 76 002 (100) + bicuculline	2	i.p.	8	7.2 ± 0.8	<0.01[c]
	+ bicuculline	2	i.p.	8	5.8 ± 0.9	NS
	+ methergoline	10	p.o.	8	4.9 ± 0.7	NS
	+ quipazine (5) + methergoline	10	p.o.	8	5.6 ± 0.8	<0.05[c]
	+ fenfluramine (3) + methergoline	10	p.o.	7	5.6 ± 0.6	<0.01[c]
	+ imipramine 5 d (10) + methergoline	10	p.o.	8	6.8 ± 0.9	<0.05[c]

[a] with respect to sham operated.
[b] with respect to bulbectomized vehicle treated.
[c] with respect to drug treated group without antagonist pretreatment.

References

CAIRNCROSS, K.D., COX, B., FORSTER, CHR. & WREN, A.F. (1979). A new model for the detection of antidepressants drugs: olfactory bulbectomy in the rat compared with existing models. *J. Pharmacological Methods*, **1**, 131–143.

LLOYD, K.G., WORMS, P., DEPOORTERE, H. & BARTHOLINI, G. (1979). Pharmacological profile of SL 76 002. A new GABA-mimetic drug. In: *GABA-Neurotransmitters, Pharmacochemical, Biochemical and Pharmacological Aspects*. Eds. P. Krögsgaard-Larsen, J. Scheel-Krüger and H. Kofod, Munksgaard, Copenhagen, pp. 308–325.

MORSELLI, P.L., BOSSI, L., HENRY, J.F., ZARIFIAN, E. & BARTHOLINI, G. (1979). Preliminary observation on the action of SL 76 002, a new GABA-mimetic compound, in neuropsychiatric disorders. *Communication at the Symposium on GABA and other inhibiting amino acids*. Myrtle Beach, South Carolina.

RIGTER, H., VAN RIEZEN, H. & WREN, A. (1977). Pharmacological validation of a new test for the detection of antidepressant activity of drugs. *Brit. J. Pharmac.*, **59**, 451P–452P.

SIECK, M.H. (1972). The role of the olfactory system in avoidance learning and activity changes. *Physiol. Behav.*, **8**, 705–710.

WONG, D.T., HORNG, J.S., BYMASTER, F.P., HAUSER, K.L. & MOLLOY, B.B. (1974). A selective inhibitor of serotonin uptake: Lilly 110140, 3-(p-trifluoromethyl phenoxy)-N-methyl-3-phenyl propylamine. *Life Sciences*, **15**, 471–479.

Duration of effect of repeated electroconvulsive shocks on plasma corticosterone responses to pharmacological stimuli in the rat

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We have previously reported that plasma corticosterone in the male rat is elevated by i.p. injections of oxotremorine (oxo-T) or 5-hydroxy-L-tryptophan (5-HTP). When rats are treated with α-methyl-*p*-tyrosine (α-MpT), they develop elevated plasma corticosterone levels which may be suppressed with i.p. clonidine (Steiner & Grahame-Smith, 1979a). Dose response curves obtained for the plasma corticosterone response to oxo-T, 5-HTP and clonidine after α-MpT in rats treated with a single electro-convulsive shock (ECS) daily for 10 days have been shown to lie to the left of curves obtained in sham-shocked controls (Steiner & Grahame-Smith, 1979b).

The time taken for the disappearance of the difference in the corticosterone response between rats treated with a course of ECS and sham-shocked controls was studied. Male rats were given 1 ECS daily for 10 days under halothane anaesthesia. Controls were anaesthetised only. One day, 3 days and 6 days after the last ECS, the plasma corticosterone response was obtained to oxo-T (0.04 mg/kg, i.p.) 5-HTP (5 mg/kg, i.p.) and clonidine (0.04 mg/kg, i.p.) after α-MpT 14–16 h earlier (400 mg/kg, i.p.).

Twenty-four hours after the last ECS, significant differences were obtained in the corticosterone response to oxo-T, 5-HTP and clonidine between ECS and sham-shocked controls. All differences had resolved 6 days after the last ECS. However it was found that the corticosterone response to 5-HTP and to clonidine in sham-shocked animals progressively increased after anaesthesia was suspended while the response in ECS animals was unchanged. In rats tested with oxo-T, there was a progressive decline in the response of the ECS treated animals and a progressive rise in the response of controls.

Dose response curves for ECS and sham-shocked animals were compared with dose response curves obtained in animals which had never been handled. The curves for animals given ECS treated with oxo-T, 5-HTP and clonidine were similar to the curves of unhandled animals. The curves for sham-shocked controls were shifted to the right.

These data do not suggest that receptor hypersensitivity occurs after repeated ECS in this model. Rather, the anaesthetic procedure appears to suppress the corticosterone response. Repeated ECS restores the suppressed response towards the response obtained in unhandled rats.

References

STEINER, J.A. & GRAHAME-SMITH, D.G. (1979a). Pharmacological control of corticosterone secretion in the intact rat. *Br. J. Pharmac.*, (In press).

STEINER, J.A. & GRAHAME-SMITH, D.G. Repeated electroconvulsive shock enhances serotoninergic stimulation and α-adrenergic inhibition of corticosterone secretion in the rat. *Proceedings of the Third European Neurosciences Meeting, September, 1979b.* (In press).

Effect of chronic treatment with neuroleptics on choline high affinity uptake and choline acetyltransferase activity in rat striatum

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Acute administration of neuroleptics decreases acetylcholine (ACh) level in the striatum (Sethy & Van Woert, 1974) and stimulates ACh turnover (Costa, Cheney, Mao & Moroni, 1978). These effects disappear after repeated administrations (Costa *et al.*, 1978).

We investigate the changes occurring in striatal choline high affinity uptake and choline acetyltransferase (CAT) activity in adult male rats, Wistar strain, treated for 45–60 days daily with oral haloperidol and pimozide or twice a week with fluspirilene i.m. All treated rats were sedated but had the same increase in body weight as the controls.

At the end of the treatment the rats were killed by decapitation, the brain rapidly removed and the striatum dissected out. Choline high affinity uptake was measured by the method of Simon, Atweh & Kuhar (1976) on a crude synaptosomal fraction. CAT activity was assayed by the micromethod of Fonnum (1975) on striatal homogenates.

The results are shown in Table 1. All three neuroleptics caused a statistically significant stimulation of choline high affinity uptake and a decrease in CAT activity. Single administration of the same doses of neuroleptics did not affect either choline uptake or

Table 1 Effect of chronic neuroleptic treatment on choline high affinity uptake (pmol/mg protein. 4 min) and CAT activity (nmol/mg protein min) in rat striatum.

Drug	Dose (mg/kg) and route	Choline uptake	P	% change	CAT activity	P	% change
Vehicle	os	7.73 ± 0.25 (11)	—	—	1.72 ± 0.01 (5)	—	—
Haloperidol	1 os	9.99 ± 0.32 (6)	<0.01	+29	1.38 ± 0.01 (5)	<0.01	−20
Pimozide	1 os	10.69 ± 0.75 (5)	<0.01	+38	1.26 ± 0.04 (5)	<0.01	−27
Fluspirilene	1 im	9.94 ± 0.86 (5)	<0.01	+29	1.00 ± 0.05 (5)	<0.01	−42

Number of rats in parenthesis.

CAT activity. Only 18% decrease in CAT activity was found after 12 days of treatment with fluspirilene. Kinetic analysis of the sodium dependent choline high affinity uptake showed a significant increase in V_{max} but no change in K_m.

These experiments demonstrate that during chronic neuroleptic treatment important adaptive changes occur in the mechanisms involved in ACh synthesis in the striatum. It is not clear however how the interplay of the two apparently contradictory changes could result in a return to normal ACh level and turnover rate.

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References

COSTA, E., CHENEY, D.L., MAO, C.C. & MORONI, F. (1978). Action of anti-schizophrenic drugs on the metabolism of γ-aminobutyric acid and acetylcholine in globus pallidus, striatum and nucleus accumbens. *Fed. Proc.*, **37**, 2408–2414.

FONNUM, F. (1975). A rapid radiochemical method for the determination of choline acetyltransferase. *J. Neurochem.*, **24**, 407–409.

SETHY, V.H. & VAN WOERT, M.H. (1974). Regulation of striatal acetylcholine concentration by dopamine receptors. *Nature, Lond.*, **251**, 528–530.

SIMON, J.R., ATWEH, S. & KUHAR, M.J. (1976). Sodium dependent high affinity choline uptake: a regulatory step in the synthesis of acetylcholine. *J. Neurochem.*, **25**, 909–922.

Regional distribution of rat brain vasoactive intestinal polypeptide sensitive adenylate cyclase: effect of neurotoxic lesions on hypothalamic enzyme activity

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Vasoactive intestinal polypeptide (VIP) is present in the CNS (Saïd & Rosenberg, 1976) and may play a role in neurotransmission (Giachetti, Saïd, Reynolds & Koniges, 1977). Recently an extensive regional distribution of the peptide has been determined with high concentrations being located in the neocortex, hippocampus, striatum and hypothalamus (Besson, Rotsztejn, Laburthe, Epelbaum, Beaudet, Kordon & Rosselin, 1979). VIP stimulates brain adenylate cyclase activity (Deschodt-Lanckman, Roberrecht & Christophe, 1977) and the regional distribution of VIP-sensitive adenylate cyclase has been investigated (Quik, Iversen & Bloom, 1978) but without prior knowledge of the distribution of the peptide. We have therefore reviewed in detail the regional activity of VIP-sensitive adenylate cyclase in homogenates of rat brain. In addition, since the hypothalamus is a site where VIP is an important neuropeptide (Emson, Fahrenkrug, Schaffalitsky de Muckadell, Jessell & Iversen, 1978), we have attempted, using lesioning studies, to define upon which neuronal elements this enzyme may reside. The methods used to study adenylate cyclase activity have been described elsewhere (Albano, Maudsely, Brown & Barnes, 1973). Adenosine-3′; 5′-monophosphate (cAMP) was measured by the method of Brown, Albano, Ekins & Sgherzi (1971).

In whole brain homogenates, VIP (0.1–10 μM) stimulated adenylate cyclase activity with maximal responsiveness at 2 μM; this stimulation being greatest after a 10 min incubation. The order of sensitivity of homogenates from different brain regions to 2 μM VIP after a 10 min incubation was as follows: olfactory

bulb > hippocampus > thalamus > occipital cortex ≃ midbrain ≃ frontal cortex ≃ hypothalamus > striatum > cerebellum ≃ brain stem > spinal cord.

In hypothalamic studies an attempt was made to study the neuronal localization of VIP-sensitive enzyme. Several groups of animals were prepared with neurotoxin-induced lesions placed stereotaxically within the mediobasal hypothalamus (co-ordinates A + 1.0, L ± 1.0, V − 8.5, Pellegrino & Cushman, 1967). One group of animals received a bilateral intrahypothalamic injection of kainic acid (2 μg in 2 μl of 50 mM phosphate-buffered saline). Another group received 6-hydroxydopamine (6-OHDA, 8 μg in 2 μl saline ascorbate vehicle) and a third group received 5,7-dihydroxytryptamine (5,7-DHT, 10 μg in 2 μl saline ascorbate vehicle). Control animals received bilateral injections of the appropriate vehicle into the same site. One week later the response of hypothalamic adenylate cyclase to 2 μM VIP was tested in the 3 lesioned groups and compared with the appropriate sham-operated controls. Prior injection of 6-OHDA significantly reduced the ability of VIP to stimulate hypothalamic enzyme activity (sham: basal = 405 ± 21, pmoles cAMP/mg protein/10 min, stimulated 681 ± 13; lesion: basal = 410 ± 13, stimulated 484 ± 14, triplicate determinations assayed in triplicate, for significances data pooled from two assays i.e. n = 6; $P < 0.05$ Mann Whitney U test). In contrast, there was no difference between sham and kainate or sham and 5,7-DHT treated animals in the ability of 2 μM VIP to stimulate hypothalamic adenylate cyclase activity. Biochemically, each of the neurotoxin lesions produced different changes in hypothalamic neurotransmitter systems. The kainate lesion produced loss of GABA containing elements, as judged by significant reduction in high-affinity GABA uptake ($P < 0.01$), and significant loss of hypothalamic dopamine concentrations (to 28% of control) with no detectable change in noradrenaline. The 5,7-DHT lesion caused damage to hypothalamic 5-hydroxytryptamine terminals as judged by 51% loss of high-affinity uptake sites for this neurotransmitter. The 6-OHDA lesion produced a loss of noradrenaline (to 18% of control) and dopamine (to 39% of control).

These results provide extended data on the regional distribution of VIP-sensitive adenylate cyclase in rat brain and point to a possible localization of the enzyme on noradrenergic neurones in the rat hypothalamus.

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References

ALBANO, J., MAUDSLEY, D.V., BROWN, B.L. & BARNES, G.D. (1973). A simplified procedure for the determination of adenylate cyclase activity. *Biochem. Soc. Trans.*, **1**, 477–479.

BESSON, J., ROTSZTEJN, W., LABURTHE, M., EPELBAUM, J., BEAUDET, A., KORDON, C. & ROSSELIN, G. (1979). Vasoactive intestinal peptide (VIP): Brain distribution, subcellular localization and effect of deafferentation of the hypothalamus in male rats. *Brain Res.*, **165**, 79–85.

BROWN, B.L., ALBANO, J., EKINS, R.P. & SGHERZI, A. (1971). A simple and sensitive saturation assay for the measurement of adenosine-3:5-monophosphate. *Biochem. J.*, **121**, 561–562.

DESCHODT-LANCKMAN, M., ROBERRECHT, P. & CHRISTOPHE, J. (1977). Characterization of VIP sensitive adenylate cyclase in guinea-pig brain. *FEBS Lett.*, **83**, 76–80.

EMSON, P.C., FAHRENKRUG, J., SCHAFFALITSKY DE MUCKADELL, O.B., JESSELL, T.M. & IVERSEN, L.L. (1978). Vasoactive intestinal polypeptide (VIP): vesicular localization and potassium evoked release from rat hypothalamus. *Brain Res.*, **143**, 174–178.

GIACHETTI, A., SAÏD, S.I., REYNOLDS, R. & KONIGES, F.C. (1977). Vasoactive intestinal polypeptide in brain: localization in and release from nerve terminals. *Proc. Natl. Acad. Sci. U.S.A.*, **74**, 3424–3428.

PELLEGRINO, L.J. & CUSHMAN, A.J. (1967). *A Stereotaxic Atlas of the Rat Brain: New York, Meredith Publishing Co.*

QUIK, M., IVERSEN, L.L. & BLOOM, S.R. (1978). Effect of vasoactive intestinal peptide (VIP) and other peptides on cAMP accumulation in rat brain. *Biochem. Pharmacol.*, **27**, 2209–2213.

SAÏD, S.I. & ROSENBERG, R.N. (1976). Vasoactive intestinal polypeptide: abundant immunoreactivity in neural cell lines and normal nervous tissue. *Science*, **192**, 907–908.

Modulation of the electrically-evoked release of [^{3}H]-histamine from the guinea-pig hypothalamus

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Dithiothreitol potentiates the depolarizing action of substance P in the frog spinal cord *in vitro*

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Substance P (sP) depolarizes motoneurons in the isolated hemisected frog spinal cord (Konishi & Otsuka, 1974). This system potentially provides a useful bioassay but initial experience showed the preparation to be less sensitive than desired. Dithiothreitol (DTT), a sulphydryl-protecting agent (Cleland, 1964), has been used to protect sP in release studies *in vitro* (Otsuka & Konishi, 1976) and so we have considered the possibility that DTT might potentiate the action of sP.

Hemisected frog (*Rana temporaria*) spinal cords were perfused (0.6 ml/min) at 14°C with frog Ringer, bubbled with 95% O_2 and 5% CO_2. DC potentials, recorded electrotonically from the most caudal ventral roots, using Ag-AgCl-agar wick electrodes, were displayed on pen recorders. In order to observe the direct effects of sP on mononeurons only, procaine hydrochloride (2 mM) was included in the Ringer. Calcium was omitted to increase sensitivity to sP (Otsuka & Yanagisawa, 1978). Some preparations were treated with collagenase (Boehringer, 1 mg/ml) for 30 min to help remove the pial membrane (Otsuka & Yanagisawa, 1978). Synthetic sP (Beckmann) was applied via the perfusion system for periods of 30 s with a long dose cycle (30 min) to avoid interaction between doses.

When DTT (10 mM, Sigma) was applied in conjunction with sP, there was a shift in the dose response curve to the left corresponding to a potentiating dose ratio of 0.26 ± 0.03 (n = 5). Trans-4,5-dihydroxy-1,2-dithiane (oxidised DTT), lacking sulphydryl-protecting properties, failed to alter the nature of responses to sP (Fig. 1b). Collagenase pre-treatment increased the rates of onset and offset of responses to sP and increased sensitivity from a threshold dose of about 0.5 μM, under normal conditions, to about 10 nM. Following collagenase pre-treatment, DTT did not potentiate sP responses (Fig. 1e).

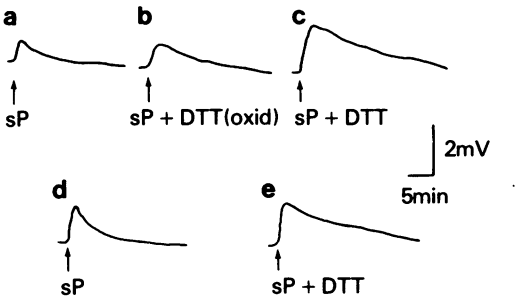

Figure 1 Depolarizing responses to a 30 s application of (a) 2.5 μM sP; (b) 2.5 μM sP with 10 mM oxidized DTT; (c) 2.5 μM sP with 10 mM DTT; (d) 1.25 μM sP; (e) 1.25 μM sP with 10 mM DTT. Responses a–c are from one preparation; responses d & e are from a collagenase (1 mg/ml) pre-treated preparation. In each case there were 30 min between doses.

These results suggest that the sP-potentiating action of DTT may be related to its sulphydryl-protecting properties. Since this potentiation was not seen when the pial membrane had been loosened by collagenase treatment, the site of action of DTT may be a membrane-bound enzyme.

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References

CLELAND, W.W. (1964). Dithiothreitol: A new protective reagent for sulphydryl groups. *Biochemistry*, **3**, 480–482.

KONISHI, S. & OTSUKA, M. (1974). The effects of substance P and other peptides on spinal neurons of the frog. *Brain Res.*, **65**, 397–410.

OTSUKA, M. & KONISHI, S. (1976). Release of substance P-like immunoreactivity, from isolated spinal cord of newborn rat. *Nature*, **264**, 83–84.

OTSUKA, M. & YANAGISAWA, M. (1978). The action of substance P on motoneurons of the isolated rat spinal cord. *Advances in Pharmacology and Therapeutics, Volume 2 Neurotransmitters*. P. Simon (Ed.), Pergamon Press, 181–190.

The effect of methoxamine on single cortical neurones

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Cortical neurones can respond both with excitation and depression to microelectrophoretically applied sympathomimetic amines. There is evidence that the excitatory responses are mediated by α-adrenoceptors and the depressant responses by β-adrenoceptors (see Szabadi, 1979). In previous experiments in this laboratory (Bevan, Bradshaw & Szabadi, 1977) it was found that the α-adrenoceptor stimulant methoxamine had purely excitatory actions on cortical neurones, however, it appeared to be much less potent than another α-adrenoceptor agonist, phenylephrine. We report here some further observations of the effects of methoxamine on cortical neurones.

Single spontaneously active neurones were studied in the cerebral cortices of halothane anaesthetised rats. Drugs were applied by microelectrophoresis. Our methods are described elsewhere (Bevan *et al.*, 1977).

Eighty-five neurones yielded consistent responses to methoxamine; all were excited by the drug. Phenylephrine was applied to 30 of these cells; in every case phenylephrine also evoked excitatory responses. The effects of noradrenaline were examined on 54 cells excited by methoxamine; 52 were excited and two depressed by noradrenaline. The effects of isoprenaline were studied on 11 cells excited by methoxamine; two were excited and nine depressed by isoprenaline.

Methoxamine had a significantly lower apparent potency than either noradrenaline (*t*-test, $P < 0.001$) or phenylephrine (*t*-test, $P < 0.001$); the mean (±s.e. mean) equipotent current ratios were 0.413 ± 0.030 (noradrenaline/methoxamine: $n = 16$) and 0.390 ± 0.096 (phenylephrine/methoxamine: $n = 7$).

The effect of methoxamine upon responses to noradrenaline was examined on 11 cells. The equilibrium change in firing rate evoked by the simultaneous application of the two drugs was compared with the sum of the equilibrium changes in firing rate evoked by the two drugs applied separately; on each cell the procedure was repeated using a control agonist, acetylcholine, in place of noradrenaline. In every case the response to methoxamine and noradrenaline applied together was smaller than the sum of the responses to the two drugs applied separately (mean reduction from the summated individual responses: $38.4\% \pm 4.7\%$). In contrast there was a significantly smaller discrepancy (*t*-test, $P < 0.01$) between the magnitude of the response evoked by the simultaneous application of acetylcholine and methoxamine and the sum of the magnitude of the individual responses to these drugs (mean reduction: $24.6\% \pm 4.4\%$).

These observations are consistent with the hypothesis that methoxamine may act as a partial agonist (cf. Ariëns, Simonis & Rossum, 1964) at the same receptors which mediate excitatory responses to noradrenaline in the cerebral cortex. Previous findings (Bevan *et al.*, 1977) indicate that these are α-adrenoceptors. Thus the action of methoxamine in the cerebral cortex may be similar to its action in ventricular myocardium, where it has been shown to be a partial agonist at α-adrenoceptors (Schümann & Endoh, 1976).

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References

ARIËNS, E.J., SIMONIS, A.M. & ROSSUM, J.M. VAN (1964). Drug-receptor interaction: Interaction of one or more drugs with one receptor system. In *Molecular Pharmacology*, ed. Ariëns, E.J., pp. 119–286. New York: Academic Press.

BEVAN, P., BRADSHAW, C.M. & SZABADI, E. (1977). The pharmacology of adrenergic neuronal responses in the cerebral cortex: evidence for excitatory α- and inhibitory β-receptors. *Br. J. Pharmac.*, **59**, 635–641.

SCHÜMANN, H.-J. & ENDOH, M. (1976). α-Adrenoceptors in the ventricular myocardium: clonidine, naphazoline and methoxamine as partial α-agonists exerting a competitive dualism in action to phenylephrine. *Eur. J. Pharmac.*, **36**, 413–421.

SZABADI, E. (1979). Adrenoceptors on central neurones: microelectrophoretic studies. *Neuropharmacology*. (In press).

The anomalous depressant activity of N^{τ}-methyl histamine

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Although the histamine metabolite N^{τ}-methyl histamine (3 MH) has negligible activity on histamine H_1- or H_2-receptors (Black, Duncan, Durant, Ganellin & Parsons, 1972) it has been reported to have appreciable depressant activity when applied to central neurones by microiontophoresis (Haas, Anderson & Hosli, 1973; Phillis, Tebecis & York, 1968). This phenomenon was therefore investigated further.

To obtain quantitative estimates of the potency of 3 MH relative to histamine (HA) male albino rats (approx. 300 g) were anaesthetized with urethane plus pentobarbitone (0.4 g and 40 mg/kg respectively i.p.) and the activity of glutamate-stimulated somatosensory cortical cells recorded, two barrels of each 7-barrelled micropipette (tip diameter 7–12 μm) containing 2 M NaCl, one for recording and the other for current balancing. Drug solutions were placed in the other barrels, including histamine dihydrochloride (0.2 M, pH 5.4) and N^{τ}-methyl histamine dihydrochloride (0.2 M, pH 4.5). HA and 3MH were applied by microiontophoresis alternately for 20 s in every 100 s and the number of spikes in the iontophoretic period recorded digitally and compared with the mean in the previous two 20 s periods. Consistent with the previous reports, both HA and 3 MH were depressant ($< = 100$ nA) on 41/43 cells, excitant on 1 and had no detectable effect on 1. The relationship between log current (35–140 nA) and response, was found to be parallel on 24/26 cells and the mean equipotent current ratio, determined graphically, was $70 \pm 7\%$ ($\pm$ s.e. mean).

To determine to what extent this might reflect differences in transport, release by microiontophoresis into saline was assayed by liquid scintillation spectrometry using similar multibarrelled pipettes and similar drug solutions to those used above but labelled (4.5–5.5 dpm/pmol) with [U-^{14}C]-histamine dihydrochloride (The Radiochemical Centre, Amersham) or N^{τ}-[^{3}H]-methyl histamine dihydrochloride (kindly provided by Dr I.R. Smith). For both compounds linear relationships were found between release and charge passed (0–100 nA, 5–10 mins) and there was no significant difference between their transport numbers, 0.152 ± 0.009 for HA and 0.139 ± 0.007 for 3 MH (mean $\pm$ s.e. mean; n, the number of electrodes examined = 13 and 11, $P > 0.05$, F-test).

It is also thought unlikely that the activity of 3 MH can be attributed totally to potentiation of endogenous HA by inhibition of histamine N-methyl transferase because when the activity of spontaneous cells was recorded with 3-barrelled microelectrodes (tip diameters 3–5 μm) out of 24 cells, 7 were sensitive only to HA (3 MH producing no detectable effect with currents up to 70 nA), 11 were insensitive to HA and 3 MH, and 6 were sensitive to both HA and 3 MH.

The possibility is therefore suggested that although HA may be released synaptically and its actions on histamine H_1- and/or H_2-receptors terminated by methylation, HA and 3 MH may also act on other receptors.

References

BLACK, J.W., DUNCAN, W.A.M., DURANT, C.J., GANELLIN, C.R. & PARSONS, E.M. (1972). Definition and antagonism of histamine H_2-receptors. *Nature*, **236**, 385–390.

HAAS, H.L., ANDERSON, E.G. & HÖSLI, L. (1973). Histamine and metabolites: their effects and interactions with convulsants on brain stem neurones. *Brain Res.*, **51**, 269–278.

PHILLIS, J.W., TEBECIS, A.K. & YORK, D.H. (1968). Histamine and some antihistamines: their actions on cerebral cortical neurones. *Br. J. Pharmac. Chemother.*, **33**, 426–440.

The inhibition of clonidine-induced sedation in the mouse by anti-depressant drugs

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The central α-adrenoceptors mediating the sedative and hypotensive actions of clonidine in the rat resemble peripheral presynaptic α-adrenoceptors in their sensitivity to α-adrenoceptor agonists and antagonists (Drew, Gower & Marriott, 1977; 1979; Hersom, Finch & Metcalfe, 1978). Certain antidepressant drugs increase the release of [^{3}H]-noradrenaline from electrically stimulated rat cortical slices by a presumed presynaptic α-adrenoceptor blocking effect (Baumann & Maître, 1975; 1977) and inhibit the hypotensive actions of clonidine (van Zweiten, 1975). It was therefore of interest to determine whether the

sedative action of clonidine, like the hypotensive action, was antagonised by antidepressant drugs.

The effects of drugs on clonidine-induced sedation and hypothermia were investigated using dose groups of 6 to 12 I.C.I. derived male albino mice (18–22 g). Drugs were administered orally $1\frac{1}{2}$ to $5\frac{1}{2}$ h prior to clonidine, 0.2 mg/kg intraperitoneally; sedative and hypothermic effects were assessed 30 min after the clonidine injection. Sedation was assessed visually using a behavioural check list and a 'blind' procedure in which the observers were unaware of the nature of the drug treatments. Core temperature was measured by inserting a thermistor probe into the oesophagus.

Yohimbine, 1.25–10 mg/kg, and piperoxan, 20–80 mg/kg, but not thyoxamine, 20–160 mg/kg, inhibited clonidine-induced sedation. Clonidine-induced hypothermia was reduced by yohimbine but not by piperoxan or thymoxamine. These results confirmed previous findings in rats that clonidine-induced sedation is separable from the hypothermia and is inhibited by α-adrenoceptor antagonists which, in peripheral tissues, act preferentially at presynaptic α_2-adrenoceptors. The antidepressant drugs, mianserin, 10–40 mg/kg, protriptyline, 20–80 mg/kg, imipramine, 10–80 mg/kg and amitriptyline, 10–80 mg/kg, and the monoamine oxidase inhibitors tranylcypromine, 2–4 mg/kg, and iproniazid, 25–200 mg/kg, reduced clonidine-induced sedation in a dose-dependent manner. Maprotiline, 160 mg/kg, reduced the sedation only slightly; lower doses were ineffective. None of the antidepressant drugs had any significant effect on clonidine-induced hypothermia.

If the central α_2-adrenoceptors mediating sedation are located presynaptically, the sedative action of clonidine could be caused by reduced noradrenaline availability at postsynaptic receptors. Antidepressants could counteract this effect by inhibiting reuptake of noradrenaline or, in the case of monoamine oxidase inhibitors by inhibiting noradrenaline breakdown. Blockade of central α_2-adrenoceptors is another possibility. The antagonism of clonidine-induced sedation in laboratory animals could thus provide a useful screening test for antidepressants. The finding that both antidepressants and selective presynaptic α_2-antagonists produce a similar blocking of clonidine-induced sedation suggests that selective central α_2-adrenoceptor antagonism could provide a new approach to finding improved antidepressant drugs.

References

BAUMANN, P.A. & MAÎTRE, L. (1975). Blockade of the presynaptic alpha receptor in rat cortex by antidepressants. *Experientia*, **31**, 726.

BAUMANN, P.A. & MAÎTRE, L. (1977). Blockade of presynaptic α-receptors and of amine uptake in the rat brain by the antidepressant mianserine. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **300**, 31–37.

DREW, G.M., GOWER, A.J. & MARRIOTT, A.S. (1977). Pharmacological characterisation of α-adrenoceptors which mediate clonidine-induced sedation. *Br. J. Pharmac.*, **61**, 468P.

DREW, G.M., GOWER, A.J. & MARRIOTT, A.S. (1979). α_2-Adrenoceptors mediate clonidine-induced sedation in the rat. *Br. J. Pharmac.*, 1979 (In press).

HERSOM, A.S., FINCH, L. & METCALFE, G. (1978). Interactions between clonidine and α-adrenoceptor antagonists. *Proc. 7th International Congress of Pharmacology*. Abstr. 2713, Oxford: Pergamon Press.

VAN ZWEITEN, P.A. (1975). Interaction between centrally acting hypotensive drugs and tricyclic antidepressants. *Arch. int. Pharmacodyn.*, **214**, 12–30.

The effects of noradrenaline, isoprenaline, vasopressin and histamine on blood flow and oxygen consumption in the colon of the anaesthetized dog

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Segments of colon weighing 45 ± 4 g (mean ± s.e. mean) were denervated, vascularly isolated and autoperfused at constant arterial pressure in seven pentobarbitone-anaesthetized dogs (18.2 to 26.6 kg). Local arterial (P_a) and venous (P_v) pressures, colonic blood flow ($\dot{Q}$) and arteriovenous oxygen difference (A-V O_2) were measured continuously; colonic vascular resistance (R) and oxygen consumption ($\dot{V}\,O_2$) were calculated as $[R = (P_a\text{-}P_v)/\dot{Q}]$ and $[\dot{V}\,O_2 = (\text{A-V}\,O_2)\cdot\dot{Q}]$ respectively. Drugs were infused directly into the cannula delivering arterial blood to the colon; the doses used caused no significant changes in P_a or in P_v. Changes in blood concentration were calculated as (infusion rate)/(blood flow).

Noradrenaline, infused i.a. in doses from 40 ng/min to 1 μg/min, evoked graded increases in colonic vascular resistance and in A-V O_2; at higher doses within this range, $\dot{V}\,O_2$ was reduced. For example, 400 ng/min noradrenaline increased the arterial blood

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concentration by 48 ± 8 ng/ml (n = 4), reduced $\dot{Q}$ from 18.6 ± 3.5 to 9.3 ± 2.0 ml/min ($P < 0.005$) and increased A-V O_2 from 5.0 ± 0.7 to 6.8 ± 0.8 ml/100 ml ($P < 0.05$). R rose by 105 ± 17% ($P < 0.02$) and $\dot{V}$ O_2 fell by 32 ± 5% ($P < 0.005$).

Isoprenaline (20 ng/min to 1 μg/min, i.a.) caused graded reductions in colonic vascular resistance and A-V O_2. $\dot{V}$ O_2 did not change significantly. For example, 200 ng/min isoprenaline increased the arterial blood concentration by 14 ± 1 ng/ml (n = 5), increased $\dot{Q}$ from 11.5 ± 1.2 to 14.9 ± 1.1 ml/min ($P < 0.05$) and reduced A-V O_2 from 5.2 ± 0.6 to 4.2 ± 0.5 ml/100 ml ($P = 0.01$). R fell by 25 ± 4% ($P < 0.05$) and $\dot{V}$ O_2 rose by 9 ± 3% ($P > 0.05$).

Vasopressin in doses from 0.5 to 10 mu/min i.a. elicited graded increases in colonic vascular resistance and A-V O_2, and reduced $\dot{V}$ O_2. Vasopressin, 2 mu/min i.a., elevated arterial blood concentration by 226 ± 14 μu/ml (n = 4), reduced $\dot{Q}$ from 12.8 ± 1.7 to 9.0 ± 0.6 ml/min ($P < 0.03$) and increased A-V O_2 from 5.2 ± 0.7 to 5.9 ± 0.7 ml/100 ml ($P = 0.05$). R rose by 42 ± 10% ($P < 0.005$) and $\dot{V}$ O_2 fell by 19 ± 4% ($P < 0.02$).

Histamine, infused i.a. in doses from 40 ng/min to 4 μg/min, caused dose-dependent falls in R and in A-V O_2 with variable changes in colonic oxygen consumption ($\dot{V}$ O_2). For example, 970 ng/min i.a., increased blood histamine levels by 78 ± 11 ng/ml (n = 6), increased $\dot{Q}$ from 10.3 ± 1.8 to 14.1 ± 2.4 ml/min ($P < 0.01$) and reduced A-V O_2 from 5.7 ± 0.6 to 4.5 ± 0.6 ml/100 ml ($P < 0.01$). R fell by 30 ± 5% ($P < 0.02$) and $\dot{V}$ O_2 by 6.7 ± 3.1% ($P > 0.10$).

Noradrenaline and vasopressin reduced colonic blood flow and oxygen consumption; qualitatively, these responses resemble those of the small intestine to much higher concentrations of the same substances (Pawlik, Shepherd & Jacobson, 1975; Shepherd, Pawlik, Mailman, Burks & Jacobson, 1976). The concentrations of vasopressin achieved in the present investigation correlate well with the levels found in the dog after stressful stimuli, including haemorrhage (Schmid, Abboud, Wendling, Ramberg, Mark, Heistad & Eckstein, 1974; Rocha e Silva & Rosenberg, 1969); it is therefore possible that these effects of vasopressin on the colon occur on pathophysiological release of the hormone.

Both vasodilators, isoprenaline and histamine, increased colonic blood flow and reduced A-V O_2; their effects on oxygen consumption were variable.

Further experiments are required to correlate the effects of these drugs on blood flow, capillary exchange, oxygen consumption and motility in the canine colon.

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References

PAWLIK, W., SHEPHERD, A.P. & JACOBSON, E.D. (1975). Effects of vasoactive agents on intestinal oxygen consumption and blood flow in dogs. *J. Clin. Invest.*, **56**, 484–490.

ROCHA E SILVA, M. & ROSENBERG, M. (1974). The release of vasopressin in response to haemmorrhage and its role in the mechanisms of blood pressure regulation. *J. Physiol. (Lond.)*, **202**, 535–557.

SCHMID, P.G., ABBOUD, F.M., WENDLING, M.G., RAMBERG, E.S., MARK, A.L., HEISTAD, D.D. & ECKSTEIN, J.W. (1974). Regional vascular effects of vasopressin: plasma levels and circulatory responses. *Am. J. Physiol.*, **227**, 998–1004.

SHEPHERD, A.P., PAWLIK, W., MAILMAN, D., BURKS, T.F. & JACOBSON, E.D. (1976). Effects of vasoconstrictors on intestinal vascular resistance and oxygen extraction. *Am. J. Physiol.*, **230**, 298–305.

The occurrence of postsynaptic α- and β-adrenoceptors in the guinea-pig gall bladder

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The guinea-pig gall bladder receives a noradrenergic innervation (Baumgarten & Lange, 1969; Davison, Al-Hassani, Crowe & Burnstock, 1978). Adrenoceptors, if present, in this tissue have not been characterized.

In the present study we have examined the effects of phentolamine and propranolol on contractile responses to exogenously applied (−)-noradrenaline, (−)-isoprenaline, and (−)-adrenaline, and to field stimulation (1 ms duration, supramaximal voltage) in the isolated gall bladder of the guinea-pig. Mature guinea-pigs of either sex were killed and the gall

bladder was removed and divided into 4 longitudinal strips. Each strip was suspended under 1 g tension (which was maintained throughout the course of the experiment) in Krebs solution, equilibrated with 5% CO_2 in oxygen. The tissues were allowed to recover for 1 h 45 min. Contractile responses were recorded isometrically.

(−)-Noradrenaline (10^{-5} M) consistently contracted strips of guinea-pig gall bladder. On the addition of (−)-isoprenaline (10^{-5} M), 33 of 40 strips tested relaxed and the others were unaffected. (−)-Adrenaline (10^{-5} M) was added to 56 preparations and predominantly caused contractions (31 preparations). (−)-Adrenaline was also observed to cause relaxations (15 preparations), contractions and relaxations (4), and to have no effect (6). In the presence of propranolol (10^{-6} M), the magnitude of the contractions produced by (−)-noradrenaline (10^{-5} M) or (−)-adrenaline (10^{-5} M) was increased. In tissues that contracted to (−)-noradrenaline or to (−)-adrenaline, it was observed that these agents induced relaxations in the presence of phentolamine (10^{-6} M). The relaxant responses to (−)-isoprenaline were unaltered and inhibited by phentolamine (10^{-6} M) and propranolol (10^{-6} M), respectively. In tissues that relaxed to 10^{-5} M (−)-adrenaline, the magnitude of relaxant responses increased in the presence of phentolamine (10^{-6} M) and reversed to contractions in the presence of propranolol (10^{-6} M). These results demonstrate the presence of postsynaptic α-adrenoceptors, which mediate contractions, and postsynaptic β-adrenoceptors, which initiate relaxations, in the guinea-pig gall bladder.

Strips of guinea-pig gall bladder contracted in response to field stimulation at 5 Hz. These responses were unaltered by phentolamine (10^{-6} M) but greatly reduced by atropine (10^{-6} M). This illustrates the predominant excitatory cholinergic innervation of this tissue as previously described by Davison & Fösel (1975). It seems likely that the residual contractions produced by field stimulation at 5 Hz in the presence of atropine (10^{-6} M) were neural in origin as these responses were abolished by tetrodotoxin (3×10^{-6} M). In the presence of atropine (10^{-6} M), the contractions produced by field stimulation at 5 Hz were unaltered by propranolol (10^{-6} M) but reduced by phentolamine (10^{-6} M). These results suggest that the nerve-mediated contractions of the guinea-pig gall bladder may, in part, be due to a release of endogenous noradrenaline which acts at postsynaptic α-adrenoceptors.

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References

BAUMGARTEN, H.G. & LANGE, W. (1969). Extrinsic adrenergic innervation of the extrahepatic biliary duct system in guinea-pigs, cats and rhesus monkey. *Z. Zellforsch*, **100**, 600–615.

DAVISON, J.S., AL-HASSANI, M., CROWE, R. & BURNSTOCK, G. (1978). The non-adrenergic, inhibitory innervation of the guinea-pig gall bladder. *Pflügers Arch.*, **377**, 43–49.

DAVISON, J.W. & FÖSEL, S. (1975). Interactions between vagus nerve stimulation and pentagastrin or secretin on the guinea-pig gall bladder. *Digestion*, **13**, 251–254.

The effect of cooling both vagal nerves on the changes in the discharge of the sympathetic efferent nerves induced by atenolol in the anaesthetised cat

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Acute administration of the β-adrenoceptor blocking drug, atenolol, in anaesthetised cats reduces the mean arterial blood pressure and the sympathetic efferent discharge (SED); and attenuates the reflex changes in SED in response to changes in blood pressure (Scott, 1978). To determine whether these effects depend on atenolol altering the discharge from cardiopulmonary receptors with afferents in the vagi the effect of removal of these afferents by cooling both vagi in the neck was investigated. If atenolol reduced the SED by increasing the discharge of vagal afferent fibres, then the increase in SED observed during cooling should be greater after giving atenolol. Cooling would also be expected to remove the attenuation of the reflex changes in SED observed after atenolol.

Eight cats were anaesthetised with chloralose (80 mg/kg i.p.) and artificially ventilated. Body temperature and the pH, pCO_2 and pO_2 of the arterial blood were monitored and maintained within normal limits. Recordings were made of the sympathetic discharge from few-fibre preparations in the lumbar, splanchnic and renal nerves. Blood pressure was artificially raised or lowered by the administration of phenylephrine (2–10 μg/kg) or glyceryltrinitrate (2–20 μg/kg)

respectively. The SED was recorded under steady state conditions over a wide range of blood pressure, both before and during a cold-induced block of both vagi to below 0°C.

Before giving atenolol, cooling both vagi in the neck resulted in an increase in the blood pressure from 105.5 ± 5.3 mmHg (mean ± s.e. mean) to 123.1 ± 6.0 mmHg ($P < 0.01$, $n = 8$) and an increase in the SED from 19.9 ± 3.7 to 38.4 ± 5.7 impulses/s ($P < 0.005$, $n = 8$). Atenolol (3 mg/kg) i.v. reduced both the blood pressure and the SED as reported previously (Friggi, Chevalier-Cholat & Bodard, 1977; Scott, 1978). At least 1 h after giving atenolol, cooling both vagi resulted in an increase in the SED from 16.2 ± 2.5 pulses/s to 22.2 ± 3.3 impulses/s ($P < 0.005$, $n = 16$). The increase in SED was significantly less after atenolol than before giving the drug ($P < 0.005$, $n = 16$). Atenolol significantly reduced the slope of the relationship between SED and blood pressure from -0.43 ± 0.17 impulses s^{-1} $mmHg^{-1}$ to -0.14 ± 0.05 impulses s^{-1} $mmHg^{-1}$ ($P < 0.05$, $n = 6$) as reported previously (Scott, 1978). However cooling both vagi had no statistically significant effect on this relationship, the slope remaining significantly lower than that obtained before giving the drug ($P < 0.05$).

It is concluded that neither the reduction in the SED nor the attenuation of the reflex changes in SED in response to changes in blood pressure observed after atenolol is due to an increased discharge from receptors whose afferent fibres lie in the vagal nerves.

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References

FRIGGI, A., CHEVALIER-CHOLAT, A.M. & BODARD, H. (1977). Effects of a beta-adrenergic blocking drug, atenolol, on efferent nerve activity in rabbits. *Experimentia*, **33**, 1207–1208.

SCOTT, E.M. (1978). The effect of atenolol on the discharge of sympathetic efferent nerves in the anaesthetized cat. *Br. J. Pharmac. Chemother.*, **64**, 394P.

In vitro characterisation of propranolol's interaction with 5-HT receptors

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It has been reported that the optical isomers of propranolol differ in their ability to antagonise 5-HT-induced contractions of both rat fundus and uterus (Schechter & Weinstock, 1974). More recently Fozard & Mobarok Ali (1978) have described the use of specific M and D tryptamine receptor models to characterise 5-HT agonists. We have now examined the potency of the isomers of propranolol against 5-HT using models specific for each type of receptor.

Two cm segments of guinea-pig distal ileum were mounted in a 5 ml organ bath containing Kreb's solution at 37°C and aerated with 95% O_2:5% CO_2. Contractions of longitudinal muscle were measured isotonically under a tension of 1 g. Addition of methy-

Table 1 Comparison of (+) and (−) propranolol *in vitro*.

Antagonist		*Agonist*	pA₂ ± s.e. mean *Ileum + Atropine*	*Ileum + Methysergide*	*Fundus + Atropine A*
Propranolol	(+)	5HT	6.17 ± 0.245	Non-competitive at 10^{-5} M a	5.63 ± 0.31
	(−)		*7.23 + 0.055***		**6.50 ± 0.089
Propranolol	(+)	Histamine	4.16 ± 0.31		
	(−)		4.71 ± 0.33		
Propranolol	(+)	D.M.P.P.		Non-competitive at 10^{-5} M a	

[a] d(+) and l(−) propranolol caused non-competitive antagonism at 10^{-5} M, but no antagonism at 10^{-6} M.
* PA_2 l-propranolol > PA_2 d-propranolol $P < 0.001$ ($n = 28$)
** PA_2 l-propranolol > PA_2 d-propranolol $P < 0.02$ ($n = 21$)
*** l-propranolol: PA_2 ileum > PA_2 fundus $P < 0.001$ ($n = 24$)

sergide (10^{-7} M) to the Kreb's solution abolished D receptor-mediated responses (Fozard & Mobarok Ali, 1978) thus providing a specific M receptor model. In other tissues atropine (10^{-7} M) was included to inhibit M receptor effects. Isolated rat fundus preparations were mounted in a similar manner with atropine (10^{-7} M) present throughout. These latter two preparations allowed the study of specific D receptor-mediated responses. The results summarised in Table 1 show that the action of (+)- and (−)-propranolol on 5-HT-induced contractions of the methysergide blocked ileum preparation (M receptor) was non-specific, as the nicotine receptor agonist dimethylphenylpiperazinium (DMPP) was antagonised to a similar extent. On the atropine-treated guinea-pig ileum preparation (D receptor) both propranolol isomers antagonised the 5-HT-induced contractions. This effect was apparently specific since higher concentrations of both isomers were necessary to antagonise histamine-induced contractions of this tissue.

The effect of (−)-propranolol on both atropine-treated guinea-pig ileum and rat fundus was competitive yielding Schild plots with slopes of 1.03 and 1.04 respectively. In contrast the (+)isomer yielded slopes in excess of 3 in both tissues which indicated that the interaction was not competitive.

We conclude that the antagonism of 5-HT by propranolol is due to a specific and competitive interaction of the (−)isomer with the D tryptamine receptor. Furthermore, the pA_2 values obtained with (−)-propranolol on the atropinised ileum and fundus, both D receptor models, differed significantly. This may reflect a difference between the D receptor populations in these two preparations.

References

FOZARD, J.R. & MOBAROK ALI, A.T.M. (1978). Receptors for 5-hydroxytryptamine on the sympathetic nerves of the rabbit heart. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **301**, 223–235.

SCHECHTER, Y. & WEINSTOCK, M. (1974). β-Adrenoceptor blocking agents and responses to adrenaline and 5-hydroxytryptamine in rat isolated stomach and uterus. *Br. J. Pharmac.*, **52**, 283–287.

ICI 118,551, a potent β_2 adrenoceptor antagonist

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Though satisfactory selective β_1 adrenoceptor antagonists are available, a potent specific β_2 adrenoceptor antagonist has not been described. Previously described β_2 antagonists butoxamine (Levy & Wilkenfeld, 1969). H35/25 (Van Deripe & Moran, 1964) and I.P.S. 339 (Imbs, Miesch, Schwartz, Velley, Leclerc, Mann & Wernmuth, 1977) lack either potency, specificity or adequate β_2 selectivity. ICI 118,551 (erythro-DL-1-(7-methylindan-4-yloxy)-3-isopropylamino-butan-2-ol) possesses a high degree of selectivity and specificity for the β_2 adrenoceptor.

The affinity of ICI 118,551 for the β_2 adrenoceptor was studied *in vitro* on guinea-pig uterus and for the β_1 adrenoceptor on guinea-pig right atrium by determining its antagonism of isoprenaline responses in these tissues. The results in comparison with propranolol are given in the table and show that ICI 118,551 has a β_2 selectivity index of 2.09 (i.e. pA_2 uterus–pA_2 atrium).

The effect of ICI 118,551 and atenolol on the chronotropic and vasodilator actions of isoprenaline were compared in pentobarbitone anaesthetized dogs. The perfusion pressure (H.L.P.P.) in the denervated hind limb, perfused at constant flow was measured as well as heart rate. Isoprenaline was given in incremental doses to maximal response and response curves plotted for H.R. and H.L.P.P. before and after ICI 118,551 (5.0 μg–2 mg/kg i.v. $n = 4$) or atenolol (0.25, 1.0 and 4.0 mg/kg i.v. $n = 4$). Dose ratios were calculated and the effective dissociation constant K^1 determined (Bilski, Robertson & Wale, 1979). The K^1 (H.L.P.P.) for ICI 118,551 was 2.1 μg/kg ± 0.01 (s.e. mean) and K^1 (H.R.) was >500 μg/kg. The corresponding figures for atenolol were K^1 (H.L.P.P.) =

Table 1 Comparison of the antagonist activity of propranolol and ICI 118,551 against various agonists expressed as pA_2 values

Tissue	*Agonist*	*Propranolol*	*ICI 118,551*
Uterus	Isoprenaline	9.13	9.26
Atrium	Isoprenaline	8.30	7.17
Ileum	Serotonin	5.78	5.84
Ileum	Acetyl choline	5.12	5.03
Ileum	Histamine	5.41	5.15

0.253 ± 0.08 mg/kg and K^1 (H.R.) = 0.046 ± 0.003 mg/kg. These observations indicate that ICI 118,551 has a much greater affinity for the vascular β_2 receptor than the cardiac β_1 receptor. ICI 118,551 has no partial agonist activity but has a membrane stabilizing action equivalent to propanolol. It is concluded that ICI 118,551 is a potent specific β_2 adrenoceptor antagonist.

References

BILSKI, A., ROBERTSON, H.H. & WALE, J.L. (1979). A study of the relationship between β-adrenoceptor blockade and intrinsic sympathomimetic activity in rats depleted of catecholamines. *Clin. and Exp. Pharm and Physiol.*, **6**, 1–9.

IMBS, J.L., MIESCH, F., SCHWARTZ, J., VELLEY, J., LECLERC, G., MANN, A. & WERNMUTH, C.G. (1977). A potent new β_2 adrenoceptor blocking agent. *Br. J. Pharmac.*, **60**, 357–362.

LEVY, B. & WILKENFELD, D.E. (1969). An analysis of selective beta receptor blockade. *European J. Pharmacol.*, **5**, 227–234.

VAN DERIPE D.R. & MORAN, N.C. (1964). Comparison of cardiac and vasodilator adrenergic blocking activity of DCI and four analogues. *Fed. Proc.*, **24**, 712.

The action of an extract of the sea anemone *Tealia felina* (*1*) on neuromuscular transmission studied *in vitro*

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Aldeen, Elliott & Sheardown (unpublished observations) isolated a biologically active fraction (Fraction II) from the whole body of the dahlia sea anemone *Tealia felina*. Fraction II reduces histamine induced contractions of the guinea-pig ileum, and has an haemolytic action. Bioassays based on the above actions provided the units of activity in which the results are expressed.

Standard *in vitro* preparations were used; the chick biventer cervicis muscle preparation (Ginsborg & Warriner, 1960), the frog rectus muscle preparation and the rat phrenic nerve-diaphragm preparation (Bullbring, 1946). A moist chamber technique was used to record action potentials from the sciatic nerve of the frog. The action potentials were amplified with a Schlumberger Solartron CX1443 oscilloscope using AC coupling and recorded photographically from the oscilloscope face. The stimuli were 1.0 V 0.1 ms duration pulses. The nerve was immersed in frog ringer solution, or in Fraction II-Ringer solution, between recording periods.

Fraction II in a bath concentration of 2.46–9.84 units/ml reduced indirectly elicited contractions of the rat phrenic nerve diaphragm preparation. The ED_{50} was 4.9 units/ml. At the ED_{50} for indirectly evoked contractions the percentage reduction of directly evoked contractions was 20%. The block was not reversible.

The chick biventer cervicis muscle preparation responded to Fraction II (0.98–7.84 units/ml) with a slowly developing contracture. Indirectly elicited twitch contractions were reduced by a maximum of 18%.

A protracted contracture was seen in the frog rectus muscle preparation on addition of 4–8 units/ml. This contracture was maintained, the muscle failing to relax after 60 min washing. There was considerable tachyphylaxis in the contracture responses to Fraction II, in the chick and frog muscle preparations, nevertheless it was possible to show that tubocurarine (0.01 mg/ml) reduced these contractures and that eserine 4 μg/ml potentiated them.

When frog sciatic nerve was immersed in Fraction II–frog Ringer solution (1.5–6 units/ml) for 30 min, there was a (40–95%) reduction in the size of the action potential and an increase in the stimulus threshold.

The results suggest that the extract has several actions on nerve-muscle preparations. It appears to exert a depolarizing action in the chick and frog muscles, by a mechanism involving nicotinic receptors. The rate of onset of the depolarization as judged by the contractures is slow in comparison to acetylcholine induced depolarization. This difference may merely reflect our inability to achieve high concentration of the Fraction II.

The depressant action on the response to direct stimulation of the rat phrenic nerve diaphragm and the action on the sciatic nerve may be a reflection of a toxic action on membranes, similar to the haemolytic action of Fraction II.

It appears that the activity of Fraction II resembles in some respects that described by Mathias, Ross & Schachter, 1960, of an unidentified protein like substance, isolated from *Actinia equina* and *Anemonia sulcata*.

References

BULBRING, E. (1946). Observations on the isolated phrenic nerve diaphragm preparation of the rat. *Br. J. Pharmac. Chemother.*, **1**, 38–61.

GINSBORG, B.L. & WARRINER, J. (1960). The isolated chick biventer cervicis nerve muscle preparation. *Br. J. Pharmac. Chemother.*, **15**, 410–411.

MATHIAS, A.D., ROSS, D.M. & SCHACHTER, M. (1960). The distribution of 5-hydroxytryptamine, tetramethylammonium, homarine and other substances in sea anemones. *J. Physiol.*, **151**, 296–311.

A re-evaluation of the behaviour of divalent cation agonists at motor nerve endings

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The motor nerve ending has traditionally been considered as an 'enzyme' for divalent cation (Me^{2+}) substrates (e.g. Ca^{2+} and Sr^{2+}). Such an interpretation implies that the magnitude of acetylcholine (ACh) secretion is directly related to the amount of substrate bound according to the law of mass action. In view of the largely unknown series of reaction intermediates that intervene between Me^{2+} binding and ACh release, it seems appropriate to test the mass action assumption (Silinsky, 1978).

The isolated cutaneous nerve-muscle preparation of the frog was studied using standard electrophysiological methods. The mean number of ACh quanta released by a nerve impulse ($\bar{m}$) was determined by conventional methods except at Me^{2+} concentrations that produced suprathreshold levels of ACh release. Under these conditions, a modification of the method of Ceccarelli & Hurlbut (1975) was employed to calculate $\bar{m}$.

Complete curves of log $[Me^{2+}]$ versus $\bar{m}$ constructed for both Ca^{2+} and Sr^{2+} in the same cell suggested either (a), that Sr^{2+} and Ca^{2+} differ both in affinity and efficacy (but see Meiri & Rahamimoff, 1971) and/or (b), that there are 'spare' Me^{2+} binding sites (Stephenson, 1956). To test for spare Me^{2+} receptors, La^{3+} (1 μM) was employed as an irreversible antagonist of depolarization-secretion coupling. The La^{3+}-mediated depression in $\bar{m}$, although not reversible, was surmountable by small (as little as 200 μM) increases in $[Ca^{2+}]$ but not surmountable by even very high (10 mM) $[Sr^{2+}]$.

The results suggest that compared to the partial agonist, Sr^{2+} (Meiri & Rahamimoff, 1971), Ca^{2+} may behave as a full agonist of high efficacy and produce maximal levels of ACh release whilst leaving a large proportion of Me^{2+} binding sites spare. The law of mass action may thus not be applicable to studies on the binding-effect relationship for Ca^{2+} at motor nerve endings.

References

CECCARELLI, B. & HURLBUT, W.P. (1975). The effects of prolonged repetitive stimulation in hemicholinium on the frog neuromuscular junction. *J. Physiol.*, **247**, 163–188.

MEIRI, U. & RAHAMIMOFF, R. (1971). Activation of transmitter release by strontium and calcium ions at the neuromuscular junction. *J. Physiol.*, **215**, 709–726.

SILINSKY, E.M. (1978). Enhancement by an antagonist of transmitter release from frog motor terminals. *Br. J. Pharmac.*, **63**, 485–493.

STEPHENSON, R.P. (1956). A modification of receptor theory. *Br. J. Pharmac.*, **11**, 379–393.

Effects of muscarinic agonists on the potassium permeability of smooth muscle

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Binding studies of radioactively labelled agonists and antagonists to muscarinic receptors have revealed two or more types of binding site (Hulme, Burgen & Birdsall, 1976; Ward & Young, 1977). Others have found that certain antagonists to have different affinities for muscarinic receptors in different tissues (Barlow, Burston & Vis, 1979). If different muscarinic receptor types exist they may be linked in different ways to the contraction produced. We have compared the relative potencies of muscarinic agonists to produce contraction and to increase the potassium per-

meability of the membrane of guinea-pig longitudinal ideal muscle. This type of experiment was also done by Burgen & Spero (1968) who found that some muscarinic agonists such as carbachol were considerably more potent at producing contraction than at increasing permeability, whereas others scarcely differed in their effects on contraction and permeability.

Strips of separated longitudinal muscle of ileum were loaded with ^{42}K by incubating for 3–5 h in physiological salt solution of normal composition containing this isotope. They were then transferred to a narrow tube at 36°C in which they were perfused at 2.2 ml/min with non-radioactive physiological salt solution containing tetrodotoxin (2×10^{-7} M) to prevent the effects of stimulating ganglia. Loss of radioactivity from the tissue was measured by collecting the perfusate. The solution bathing the muscle was changed to one containing a muscarinic agonist and the resulting contraction and increase in ^{42}K efflux rate constant were measured. The latter was used as an estimate of the increase in the potassium permeability of the smooth muscle membrane.

We compared the ratio of the ED_{50} for contraction with the ED_{50} for the increase in membrane permeability for a number of muscarinic agonists (acetylcholine, carbachol, methylfurmethide and tetramethylammonium). We found carbachol, for example, to be 13 times more potent at producing contraction than in increasing membrane permeability. Methylfurmethide was 15 times more potent. These results are somewhat surprising since Burgen & Spero (1968) found the ratios for carbachol and methylfurmethide to be very different—332 and 5 respectively. Experiments were done by exposing the muscle to different concentrations of agonist for a sufficient period (1.5–4 min) for the ^{42}K efflux rate constant to reach a peak value. This value was used to plot dose response curves. The method is different from that used by Burgen & Spero (1968) and may contribute to the discrepancies between our results.

It was noticeable in our experiments that the maximum increase in ^{42}K efflux obtainable with acetylcholine was usually substantially less than with stable analogues. A similar tendency for the maximum contraction to be less with acetylcholine was also found. Nevertheless, in experiments to date, acetylcholine was found to be 14 times more potent in producing contraction than in increasing membrane permeability. We suggest differences between acetylcholine and the stable analogues are caused by the activity of cholinesterases in the preparation. The similar effects of the agonists we studied on contraction and permeability do not provide any support for the existence of more than one type of functional muscarinic receptor in this tissue.

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References

BARLOW, R.B., BURSTON, K.N. & VIS, A. (1979). Three types of muscarinic receptors? *Leeds Meeting of British Pharmacological Society.*

BURGEN, A.S.V. & SPERO, L. (1968). The action of acetylcholine and other drugs on the efflux of potassium and rubidium from smooth muscle of the guinea-pig intestine. *Br. J. Pharmac.*, **34**, 99–115.

HULME, E.C., BURGEN, A.S.V. & BIRDSALL, N.J.M. (1976). Interactions of agonists and antagonists with the muscarinic receptor. *Colloques d'INSERM*, **50**, 49–69.

WARD, D. & YOUNG, J.M. (1977). Ligand binding to muscarinic receptors in the intact longitudinal muscle strips from guinea-pig intestine. *Br. J. Pharmac.*, **61**, 189–197.

Pharmacological studies on para-aminoclonidine

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Para-aminoclonidine decreased blood pressure in anaesthetized rats when injected intracerebroventricularly (i.c.v.), however, unlike clonidine, it failed to produce such a response when administered intravenously (i.v.) (Rouot, Leclerc, Bieth, Wermuth & Schwartz, 1978). In rat aortic strips and in the pithed rat preparation, para-aminoclonidine was more potent than clonidine as an α-adrenoceptor agonist (Rouot *et al.*, 1978).

The aim of this investigation was to obtain information on possible blood pressure lowering action of para-aminoclonidine when administered peripherally. Furthermore, the cardiac presynaptic and vascular corticogram modifications induced by this compound were studied and compared with clonidine.

Blood pressure and heart rate effects of clonidine and para-aminoclonidine (0.3–5.0 μg/kg) administered either i.v. or i.c.v. were studied in normotensive male rats (C. River, Sprague Dawley, weighing 250–300 g) anaesthetized with sodium pentobarbitone (55 mg/kg, i.p.). Their potency as stimulants of cardiac presynaptic and vascular postsynaptic α-adrenoceptors was

determined in the pithed rat, in which a sustained tachycardia was induced by stimulation of the thoracic spinal cord (Cavero, Gomeni, Lefevre-Borg & Roach, 1980). The antihypertensive effects of clonidine and para-aminoclonidine (0.5 mg/kg p.o.), were studied in spontaneously hypertensive rats (Okamoto strain, over 6 months old) in which mean blood pressure was directly measured with a catheter in the tail artery. In order to assess the sedative effects of clonidine (0.1 mg/kg i.p.) and para-aminoclonidine (3.0–10.0 mg/kg i.p. and i.v.) the electrocorticogram was recorded in normotensive rats immobilized with alcuronium (1.0 mg/kg i.p.) and instrumented with monopolar recording electrodes in the cerebellum (reference electrode), the fronto- and occipito-parietal regions.

In pentobarbital anaesthetized rats, para-aminoclonidine (0.3–5.0 μg total) injected into the lateral cerebral ventricle was, at least, three times more potent than clonidine in producing a fall in carotid blood pressure, during the 60 minutes following its administration. However, both compounds exerted a similar degree of bradycardia following i.c.v. and i.v. administration. This bradycardia was almost entirely dependent on an intact and operative sympathetic drive to the cardiac pacemaker, since after pretreatment of the rats with propranolol the bradycardia was absent.

I.v. administration of clonidine, unlike para-aminoclonidine, reduced blood pressure in normotensive anaesthetized rats. However, both compounds (0.5 mg/kg, p.o.) produced an antihypertensive effect in the spontaneously hypertensive rat, the onset of action of clonidine being faster than para-aminoclonidine.

In pithed normotensive rats, para-aminoclonidine was more potent than clonidine as a stimulant of cardiac presynaptic and vascular postsynaptic α-adrenoceptors.

Para-aminoclonidine (3.0–10.0 mg/kg, i.p. or i.v.) was over 100 times less effective than clonidine (0.1 mg/kg, i.p.) in inducing sleep spindles in the electrocorticogram recorded from the fronto-parietal (sensorimotor) region of the rat immobilized with alcuronium. Furthermore, both compounds produced hypersynchronization (theta-waves) on the electrocorticogram from the occipitoparietal (visual) region (Depoortere, Honore & Jalfre, 1977).

These results indicate that both the centrally (i.c.v. studies) and peripherally (i.v.) mediated effects of clonidine are also found with para-aminoclonidine. The lack of blood pressure lowering effects of para-aminoclonidine when given intravenously to normotensive anaesthetized rats may be due to its poor penetration into the central nervous system rather than its potent short lasting vasoconstrictor effects.

References

CAVERO, I., GOMENI, R., LEFEVRE-BORG, F. & ROACH, A.G. (1980). Comparison of mianserin with desipramine, maprotiline and phentolamine on cardiac presynaptic and vascular postsynaptic α-adrenoceptors and noradrenaline re-uptake in pithed normotensive rats. *Br. J. Pharmac.* (In press).

DEPOORTERE, H., HONORE, L. & JALFRE, M. (1977) EEG effects of various imidazoline derivatives in experimental animals. In: 3rd Europ. Congr. Sleep Res., Montpellier 1976, 358–361 (*Sleep* 1976, Ed: Koella W. P. & Levin P., Karger, Basel 1977).

ROUOT, B., LECLERC., G., BIETH, N., WERMUTH, C. G., & SCHWARTZ, J. (1978). Etude pharmacologique de la para-aminoclonidine, sympathomimetique alpha. *Comptes Rendus Acad. Sci. Paris.*, **286**, 909–912.

Hypotensive response to FLA-136 by selective stimulation of central α-autoreceptors in the rat

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The clonidine analogue, FLA-136 (4-amino-3(2,6-dichlorobenzyl-idenehydrazine)-1,2,4-triazol) lowers blood pressure in hypertensive rats (Eriksson & Florvall, 1976). Like clonidine FLA-136 decelerates noradrenaline turnover in rat brain by stimulation of α-adrenoceptors sensitive to yohimbine but, unlike clonidine, does not stimulate central or peripheral post-synaptic α-adrenoceptors (Anden & Grabowska, 1977). FLA-136 therefore possesses selective α-agonist properties and we have examined the nature of the α-adrenoceptors involved in the mediation of the cardiovascular effects of FLA-136, and clonidine, following intracerebroventricular (i.c.v.) administration, to pentobarbitone-anaesthetised rats. The pre- and post-synaptic effects of both drugs in the periphery have been examined in pithed rats.

In 6 anaesthetised rats, FLA-136 (10 μg i.c.v.) reduced diastolic blood pressure (DBP) and heart rate by 24 ± 6% and 9 ± 3% (mean ± s.e. mean) respectively 6 min after dosing (initial values 110 ± 1 mm Hg and 402 ± 1 bts/min) whilst clonidine 1 μg i.c.v., caused similar hypotension and bradycardia 8 min after dosing. Yohimbine (100 μg i.c.v.) almost abolished the cardiovascular effects of FLA-136 (10 and 100 μg i.c.v.) and of clonidine (1 μg i.c.v.). In 6 animals pretreated orally with prazosin (Cavero & Roach, 1978). FLA-136 (10 μg i.c.v.) reduced DBP by 33 ± 5% and heart rate by 13 ± 2% whilst in 6 vehicle-pretreated animals DBP and heart rate fell by 34 ± 4% and 15 ± 3% respectively. Clonidine (1 μg i.c.v.) reduced DBP and heart rate by 26 ± 4% and 14 ± 1% respectively in 6 vehicle-pretreated rats but by only 10 ± 4% and 8 ± 2% in 6 prazosin-pretreated rats. Pretreatment with 6-hydroxydopamine (3 × 250 μg i.c.v.), reduced the cardiovascular effects of FLA-136 (10 μg i.c.v.).

In groups of 6 pithed rats FLA-136 (0.3 and 3 mg/kg i.v., or 10 mg/kg orally 2–3 h previously) had no effect on the frequency dependent pressor responses evoked by electrical stimulation (supramaximal voltage for 20 s at 0.25–8 Hz and pulse width 0.5 ms) of the spinal sympathetic outlow though the maximum heart rate increases were depressed. As shown by others (Doxey & Everitt, 1977) clonidine (30 μg/kg i.v.) inhibited the pressor responses and tachycardia evoked by low frequencies of stimulation. After i.v. injection, clonidine, but not FLA-136, caused an immediate pressor response.

In summary, since the cardiovascular effects of i.c.v. FLA-136 in the rat are reduced by yohimbine and 6-hydroxydopamine, but not by prazosin, FLA-136 is probably acting as a selective stimulant of central α-autoreceptors. In addition to yohimbine, prazosin antagonised the cardiovascular effects of i.c.v. clonidine indicating that central post-synaptic α-adrenoceptors are also involved in the clonidine-induced effects. Unlike clonidine, FLA-136 lacks agonist activity at peripheral pre- and post-synaptic α-adrenoceptors. Since FLA-136 selectivity stimulates central α-autoreceptors, there may be differences between these receptors and peripheral pre-synaptic α_2-adrenoceptors in the rat.

References

ANDEN, N.-E. & GRABOWSKA, M. (1977). FLA-136: Selective agonist at central α-adrenoceptors mediating changes in the turnover of noradrenaline. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **298**, 239–243.

CAVERO, I. & ROACH, A.G. (1978). The effects of prazosin on the clonidine induced hypotension and bradycardia in rats and sedation in chicks. *Br. J. Pharmac.*, **62**, 468P–469P.

DOXEY, J.C. & EVERITT, J. (1977). Inhibitory effects of clonidine on responses to sympathetic nerve stimulation in the pithed rat. *Br. J. Pharmac.*, **61**, 559–566.

ERIKSSON, H.E. & FLORVALL, L.G. (1976). The preparation and anti-hypertensive activity of 4-amino-3-(2,6-dichlorobenzylidenehydrazino)-1,2,4-triazol and some related compounds. *Acta Pharmaceut. Suec.*, **13**, 79–96.

Inhibition of noradrenergic neurotransmission by dipropyldopamine through activation of presynaptic dopamine receptors

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Evidence for the existence of inhibitory dopamine receptors on post-ganglionic sympathetic nerve terminals which modulate the stimulation-evoked release of noradrenaline has been obtained *in vitro* and *in vivo* in many noradrenergically innervated tissues (Enero & Langer, 1975; Hope, McCulloch, Story & Rand, 1977; Long, Heintz, Cannon & Kim, 1975; Lokhandwala & Buckley, 1977; Langer & Dubocovich, 1979). Whilst this presynaptic dopamine receptor has not as yet an ascribed physiological role, a recent study by Lokhandwala & Jandhyala (1979), in dogs pretreated with a ganglionic blocking agent, suggested that the presynaptic effects of dopamine play a major role in the hypotensive and renal vasodilator action of this compound.

The cardiovascular effects of dopamine are complex because of the simultaneous stimulation of alpha and beta-adrenoceptors (Goldberg, 1972). N,N-di-n-propyldopamine (DPD) is a dopamine agonist lacking β-adrenoceptor activity and having reduced alpha-adrenoceptor stimulant effects when compared to dopamine (Kohli, Goldberg, Volkman & Cannon, 1978). It was therefore considered of interest to investigate the role of presynaptic inhibitory dopamine receptors in the pharmacological effects of DPD, *in vitro* and *in vivo*.

In the isolated perfused cat spleen prelabelled with [^{3}H]-noradrenaline, DPD (0.01 to 1 μM) did not

affect the spontaneous release of radioactivity but decreased, in a concentration dependent manner, the stimulation-evoked release of neurotransmitter (1 Hz, 5 min, supramaximal voltage). In the presence of cocaine (29 μM) DPD (0.1 μM) significantly decreased the stimulation-evoked release of noradrenaline (S_2/S_1: 0.37 ± 0.04, $n = 6$, $P < 0.001$ when compared with control, S_2/S_1: 0.98 ± 0.04, $n = 4$). This effect was antagonized by (S-R)-sulpiride (1 μM). DPD (0.1 μM) significantly inhibited the stimulation-evoked [^{3}H]-noradrenaline release when both cocaine (29 μM) and phentolamine (1 μM) were added to the perfusion medium (S_2/S_1: 0.66 ± 0.04, $n = 6$, $P < 0.005$ when compared with control S_2/S_1: 0.86 ± 0.04, $n = 9$). The inhibition obtained with DPD in the presence of phentolamine was less marked than that obtained in the absence of this drug ($P < 0.001$), but it was completely antagonized by (S-R)-sulpiride. These results suggest that DPD activates presynaptic dopamine receptors although an effect on presynaptic alpha adrenoceptors probably also contributes to the decrease in neurotransmission.

Infusions of DPD (50 and 200 μg kg^{-1} min^{-1} i.v.) into pentobarbitone anaesthetized dogs reduced mean blood pressure (control values of 140 ± 2 mm Hg) by 30 ± 4 and 38 ± 5 mm Hg at the low and high infusion rates respectively ($P < 0.005$, $n = 5$). After ganglionic blockade (chlorisondamine, 2 mg kg^{-1} i.v. plus atropine, 1 mg kg^{-1} i.v.) the resting mean blood pressure was reduced to 100 ± 12 mm Hg and the hypotensive action of DPD, was abolished, indicating a neurogenic component in the hypotensive action of this compound.

In addition close arterial infusions of DPD (6.25 μg kg^{-1} min^{-1}) into the canine left renal artery increased slightly but significantly renal blood flow from a control value of 94 ± 2 ml min^{-1} to 113 ± 4 ml min^{-1}, $P < 0.05$, $n = 3$). This infusion rate of DPD antagonized the reductions in renal blood flow effected by low frequency (0.5–4 Hz, 1 msec, 10 V) stimulation of the renal sympathetic nerves, had little effect on systemic blood pressure and heart rate but did slightly reduce responses to intra renal artery injections of noradrenaline. (S-R)-sulpiride (0.5 mg/kg i.v.) antagonized but did not completely abolish this inhibitory effect of DPD on renal nerve stimulation.

Our results indicate that DPD stimulates presynaptic dopamine receptors both *in vitro* and *in vivo*. In addition it can be concluded that inhibitory presynaptic dopamine receptors play a major role in mediating the hypotensive effect of DPD and contribute, together with postsynaptic dopamine receptor activation, to the renal dilator action of this compound in the dog.

References

ENERO, M.A. & LANGER, S.Z. (1975). Inhibition by dopamine of [^{3}H]-noradrenaline release elicited by nerve stimulation in the isolated cat's nictitating membrane. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **296**, 229–247.

GOLDBERG, L.I. (1972). Cardiovascular and renal actions of dopamine: Potential clinical applications. *Pharmacol. Rev.*, **24**, 1–29.

HOPE, W., MCCULLOCH, M.W., STORY, D.F. & RAND, M.J. (1977). Effects of pimozide on noradrenergic transmission in rabbit isolated ear arteries. *Europ. J. Pharmacol.*, **46**, 101–111.

KOHLI, J.D., GOLDBERG, L.I., VOLKMAN, P.H. & CANNON, J.G. (1978). N,N-di-n-propyldopamine: A qualitatively different dopamine vascular agonist. *J. Pharmacol. exp. Therap.*, **207**, 16–22.

LANGER, S.Z. & DUBOCOVICH, M.L. (1979). Physiological and pharmacological role of the regulation of noradrenaline release by presynaptic dopamine receptors in the peripheral nervous system. In *Peripheral dopaminergic receptors* (J.L. Imbs & J. Schwartz, eds.), pp. 233–245. Pergamon Press, Oxford and New York.

LOKHANDWALA, M.F. & BUCKLEY, J.P. (1977). Presynaptic dopamine receptors as mediators of dopamine-induced inhibition of neurogenic vasoconstriction. *Europ. J. Pharmac.*, **45**, 305–309.

LOKHANDWALA, M.F. & JANDHYALA, B.S. (1979). The role of the sympathetic nervous system in the vascular actions of dopamine. *J. Pharmacol. Exp. Therap.* (In press).

LONG, J.P., HEINTZ, S., CANNON, J.G. & KIM, J. (1975). Inhibition of the sympathetic nervous system by 5,6-dihydroxy-2-dimethylamino tetralin (M-7), apomorphine and dopamine. *J. Pharm. Exp. Therap.*, **192**, 336–342.

Effects of acutely and chronically administered desipramine and mianserin on the clonidine-induced decrease in rat brain 3-methoxy-4-hydroxyphenylethyleneglycol sulphate content

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3-Methoxy-4-hydroxyphenylethyleneglycol sulphate (MOPEG) is the major metabolite of noradrenaline (NA) in rat brain. The chronic administration (10 mg kg^{-1} day^{-1}) of either desipramine or mianserin for 14 days resulted in elevated MOPEG levels in the brain of rats killed 24 h after the last injection of antidepressant (Sugrue, 1979). *In vitro* studies have shown mianserin to be an inhibitor of presynaptic alpha-adrenoceptors (Baumann & Maitre, 1977) and a decrease in the sensitivity of such receptors-in rat atrial strips to NA has been observed following the chronic administration of desipramine (Crews & Smith, 1978). The possibility that the observed elevation in MOPEG levels may be a consequence of drug-induced effects on presynaptic alpha-adrenoceptors was investigated by studying the effects of acutely and chronically administered desipramine or mianserin on the clonidine-induced decrease in the concentration of MOPEG in rat brain. The dose of clonidine used (0.1 mg/kg, i.p.) was one which has been shown neurochemically (Andén, Grabowska & Strömbom, 1976) and behaviourally (Delini-Stula, Baumann & Buch, 1979) to preferentially activate presynaptic alpha-adrenoceptors in rat brain.

Male Sprague-Dawley rats were used and MOPEG levels determined spectrophotofluorometrically (Meek & Neff, 1972). In both acute and chronic experiments the dose of desipramine or mianserin used was 10 mg/kg i.p. and the time between injection of clonidine and death was three hours.

At 30 and 60 min after acute mianserin administration brain MOPEG levels were increased by 23% and 34% respectively. Levels were unchanged 30 min after desipramine administration but were decreased by 18% at 60 minutes. Rat brain MOPEG content was 70% of control 3 h after clonidine administration. The ability of clonidine to decrease brain MOPEG levels was antagonized by 30 min pretreatment with mianserin. In contrast, 30 min pretreatment with desipramine was devoid of effect on the clonidine-induced fall.

In chronic experiments rats were injected with mianserin once daily for 14 days or once daily with desipramine for 5, 9 or 15 days. Thirty min after the last injection rats were either killed for brain MOPEG determinations, or were injected with clonidine. MOPEG levels were increased 22% after chronic mianserin. The daily administration of desipramine for 9 or 15 days elevated levels by 27% and 29% respectively. Levels were unchanged after 5 days of desipramine treatment. The ability of clonidine to lower brain MOPEG content was unaltered by any of the antidepressant administration schedules.

These results show, that in contrast to the chronic situation, acutely administered desipramine decreases rat brain MOPEG levels. In addition, the ability of acutely administered mianserin to antagonize the decrease in rat brain MOPEG content elicited by clonidine, at a dose considered to be preferentially acting on presynaptic alpha-adrenoceptors, is absent following the chronic administration of the drug. This raises the possibility that the chronic administration of mianserin may be associated with adaptive changes in the sensitivity of presynaptic alpha-adrenoceptors.

References

ANDÉN, N.-E., GRABOWSKA, M. & STROMBÖM, U. (1976). Different alpha-adrenoceptors in the central nervous system mediating biochemical and functional effects of clonidine and receptor blocking agents. *Naunyn-Schmiedebergs Arch. Pharmac.*, **292**, 43–52.

BAUMANN, P.A. & MAITRE, L. (1977). Blockade of presynaptic α-receptors and of amine uptake in the rat brain by the antidepressant mianserine. *Naunyn-Schmiedebergs Arch. Pharmac.*, **300**, 31–37.

CREWS, F.T. & SMITH, C.B. (1978). Presynaptic alpha-receptor subsensitivity after long-term antidepressant treatment. *Science.* **202**, 322–324.

DELINI-STULA, A., BAUMANN, P. & BUCH, O. (1979). Depression of exploratory activity by clonidine in rats as a model for the detection of relative pre- and post-synaptic central noradrenergic receptor selectivity of α-adrenolytic drugs. *Naunyn-Schmiedebergs Arch. Pharmac.* **307**, 115–122.

MEEK, J.L. & NEFF, N.H. (1972). Fluorometric estimation of 4-hydroxy-3-methoxyphenylethyleneglycol sulphate in brain. *Br. J. Pharmac.*, **45**, 435–441.

SUGRUE, M.F. (1979). Effects of chronic antidepressant administration on the synthesis of monoamines in rat brain. *Br. J. Pharmac.* **67**, 435–436.

High-affinity binding of [^{3}H]-imipramine to human platelets: comparison with the binding in rat brain

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The high-affinity specific binding of [^{3}H]-imipramine to membranes prepared from various regions of rat brain has been shown to be unrelated either to the mechanisms of uptake of monoamines or to any of the pharmacologically well-characterised receptors (Raisman, Briley & Langer, 1979a, b; Briley, Langer & Raisman, 1980). In addition there is a possible correlation between the clinically effective dose of tricyclic antidepressants and the concentrations required to inhibit [^{3}H]-imipramine binding (Raisman, Briley & Langer, unpublished results).

The disturbances in monoamine function found in affective disorders such as schizophrenia and depression are reflected in changes in monoamine uptake by platelets in patients suffering from these disorders (Stahl, 1977). We describe here that human platelets possess specific high-affinity binding sites for [^{3}H]-imipramine, apparently similar to those already studied in rat brain.

Platelet-rich plasma was prepared from blood obtained from healthy volunteers (age 24–35) by differential centrifugation. The platelets were lysed and homogenised and the resulting membrane suspension washed and finally resuspended in Tris/HCl, 50 mM; NaCl, 120 mM; KCl, 5 mM, pH 7.5.

[^{3}H]-Imipramine binding was carried out as described for the central nervous system (Raisman, Briley & Langer, 1979a, b). The specific binding was defined as that inhibited in the presence of desipramine (100 μM) and represented 60% of the total binding at [^{3}H]-imipramine (5 nM) with membranes from rat cerebral cortex and 70% of the total binding with human platelet membranes.

High-affinity [^{3}H]-imipramine binding in human platelets had a dissociation constant, Kd, of 1.5 ± 0.4 nM (mean of 8 donors ± s.e. mean) as compared with 4.0 ± 0.5 nM ($n = 13$) for the rat cerebral cortex. Human platelets have a greater density of binding sites than rat cortex; 668 ± 100 ($n = 8$) fmoles/mg protein and 249 ± 23 ($n = 13$) fmoles/mg protein respectively.

Inhibition of [^{3}H]-imipramine binding by a series of 21 neuroactive drugs showed that they had very similar affinities for the rat brain binding sites. A correlation between the IC_{50} values in the two tissues gave a correlation coefficient, r, of 0.895 ($P < 0.001$).

Effective clinical doses for tricyclic antidepressant drugs show considerable variation. Nevertheless, taking the means of the mid-range values from a large number of studies we find a significant correlation between mean effective clinical dose and the IC_{50} values for the inhibition of [^{3}H]-imipramine binding to platelets. For a series of 9 tricyclic antidepressants the correlation coefficient, r, is 0.820 ($P < 0.01$).

The discovery of [^{3}H]imipramine binding sites in rat brain provided a new tool for the study of the action of antidepressant drugs. The existence of these sites in human platelets increases considerably the flexibility of this tool allowing comparative studies between depressed and normal subjects and providing potential assistance in the diagnosis and pharmacology of depression.

References

BRILEY, M., LANGER, S.Z. & RAISMAN, R. (1980). Specific [^{3}H]-imipramine binding in rat brain. *Br. J. Pharmac.* (In press).

RAISMAN, R., BRILEY, M. & LANGER, S.Z. (1979a). High-affinity [^{3}H]-impramine binding in rat cerebral cortex. *Europ. J. Pharmacol.* **54**, 307–308.

RAISMAN, R., BRILEY, M. & LANGER, S.Z. (1979b). Specific tricyclic antidepressant binding sites in rat brain. *Nature* (In press).

STAHL, S.M. (1979). The human platelet: a diagnostic and research tool for the study of biogenic amines in psychiatric and neurologic disorders. *Arch. Gen. Psychiat.*, **34**, 509–520.

Hydralazine possibility of interaction with purines released from sympathetic nerve terminals

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We have shown previously that hydralazine (HYD) relaxes the tail artery by a direct effect on vascular smooth muscle cells. One of the unexpected findings of this work was the indication of the existence of a modulation of the postsynaptic actions of HYD by the sympathetic nerve terminals remaining in the tissue. Preliminary results suggested strongly that the possibility should be considered that ATP or some related purine, released from the nerve endings, could modify the inhibitory effect of HYD on smooth muscle (Worcel, 1978). In order to test this hypothesis, we examined the action of different exogenous purines on the isolated rat tail artery, denervated by *in vitro* pretreatment with 6-hydroxydopamine (6-OHDA) (Aprigliano & Hermsmeyer, 1976; Worcel, 1978). Under these conditions ATP, adenosine and inosine reduced the maximal responses to HYD in a dose dependent manner. 2-Cl-adenosine, which is resistant to tissue deaminases, is active at rather low concentrations. The dose effect curve for this purine is very steep, practically a mirror image of the dose-response curve to HYD. It is interesting to note the very low ID_{50} to 2-Cl-adenosine: 1.5×10^{-9} M. The potency ratio of the purine compounds used is 2-Cl-adenosine > adenosine > ATP > inosine.

It has been shown that theophylline, at concentrations lower than 10^{-4} M, blocks competitively the vasodilatatory actions of adenosine in coronary arteries (Bunger, Haddy & Gerlach, 1975), as well as the activation of other purinergic P_1 receptors (Burnstock, 1978). We have exposed innervated proximal segments of the rat tail arteries (not pretreated with 6-OHDA) to concentrations of 2×10^{-5} and 5×10^{-5} M theophylline which do not relax the vascular smooth muscle. Theophylline potentiates the effects of HYD on the proximal segments of the artery which are usually poorly responsive to the antihypertensor drug (Worcel, 1978). Theophylline (5×10^{-5} M) mimics the action of the denervation with 6-OHDA suggesting that the amounts of nucleotides released under our experimental conditions may be sufficient to block the postsynaptic effects of HYD. A more direct confirmation of this proposition would be provided by a direct measurement of ATP content or release from sympathetic nerve terminals in the artery. Being technically impossible by the moment to perform this measurement, we decided to evaluate indirectly purine content in the nerves, by actually measuring noradrenaline content in the artery. Indeed, it has been shown that noradrenaline and purines are associated in fixed amounts in the nerve vesicles (de Potter, 1971). It has been observed previously that there is a gradient of response to HYD in arteries from normotensive Wistar rats (NW), the proximal segments being poorly relaxed, HYD action being more manifest in distal segments. This peculiar pattern of response correlates well with the gradient of noradrenaline (NA) content in the same NW: proximal segments ($n = 8$) 4581 ± 385 pg/mg wet wt., distal segments ($n = 8$) 2709 ± 308, $P < 0.05$.

In conclusion, the suppresive effects of exogenous purines, the potentiating action of theophylline, the correlation of HYD gradient of response and the gradient of NA content seem to suggest strongly that HYD interacts at the level of smooth muscle, with endogenous-nerve released-purines. HYD appears to act on a receptor for a natural substance, the more likely candidate being adenosine. This unusual mechanism may explain the localisation of the response to HYD, limited to small arteries and arterioles. This specificity could be determined by the variation in the density of specific receptors, and by the degree of activity of sympathetic nerves.

References

APRIGLIANO, O. & HERMSMEYER K. (1976). *In vitro* denervation of the portal vein and caudal artery of the rat. *J. Pharmacol. Exp. Ther.*, **198**, 568–677.

BUNGER, R., HADDY, F.J. & GERLACH, E. (1975). Coronary responses to dilating substances and competitive inhibition by theophylline in the isolated perfused guinea-pig heart. *Pflügers Arch.*, **358**, 213–224.

BURNSTOCK, G. (1978). A basis for distinguishing two types of purinergic receptor, in *Cell membrane receptors for drugs and hormones: a multidisciplinary approach.* Ed. by R.W. Straub & L. Bolis, Raven Press, New York, pp. 107–118.

DE POTTER, W.P. (1971). Noradrenaline storage particles in the splenic nerves. *Phil. Trans. Roy. Soc. (London)* **B 261**, 313–317.

WORCEL, M. (1978). Relationship between the direct inhibitory effect of hydralazine and propildazine on arterial smooth muscle contractility and sympathetic innervation. *J. Pharmacol. Exp. Ther.*, **207**, 320–330.

The mechanism of action of ATP on the rat anococcygeus muscle

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The non-adrenergic transmitter of inhibitory neurones in the rat anococcygeus muscle (Gillespie, 1972) is still unidentified. Burnstock, Cocks & Crowe (1978) suggested that it is ATP. An objection to this view is that ATP often causes contraction of the rat anococcygeus *in vitro* in circumstances where field-stimulation of the inhibitory neurones causes relaxation. Burnstock *et al.* (1978) sought to explain this by proposing that contractions were due to ATP induced prostaglandin release and they presented traces showing that the prostaglandin synthesis inhibitor, indomethacin, changes contractile responses to ATP into relaxations.

We have also sought to test this hypothesis and argue that if ATP causes prostaglandin release in the presence of a spasmogen, the prostaglandin thus masking any direct relaxant effect of ATP, then ATP should also cause the release of prostaglandin in the absence of spasmogen and that pre-treatment with indomethacin will inhibit contractile responses to ATP.

Anococcygeus muscles were set up in Krebs solution under 1 g resting tension at 37°C. Responses were recorded isometrically. Frequency effect curves were elicited to field stimulation of noradrenergic nerves (0.5 ms, 50 V) and compared with concentration effect curves to ATP and noradrenaline both before and after 60 min incubation with indomethacin (5.6 μM). One muscle from each animal acted as a concurrent control for the other (test) tissue and was given vehicle (ethanol 4 μl) in place of indomethacin.

Responses to field stimulation and ATP were depressed after treatment with indomethacin; responses to noradrenaline were not appreciably altered. Part of the depression was due to the vehicle (ethanol 0.02% in bath), but as contractions induced by ATP were not abolished by indomethacin (5.6 μM) we conclude that ATP induces contractions of the rat anococcygeus muscle *in vitro* by a direct mechanism and not by stimulating prostaglandin synthesis.

References

BURNSTOCK, G., COCKS, T. & CROWE, RAHIMA (1978). Evidence for purinergic innervation of the anococcygeus muscle. *Br. J. Pharmac.*, **64**, 13–20.

GILLESPIE, J.S. (1972). The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Br. J. Pharmac.*, **45**, 404–416.

Effects of dopamine and related drugs on the plexus-containing longitudinal muscle preparation of the guinea-pig ileum

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The presence of dopamine (DA) in significant quantities in the peripheral sympathetic nerves is well-documented (Bjorklund, Cegrell, Falck, Ritzen & Rosengren, 1970) and it has been suggested by Thorner (1975) that DA exerts specific actions as a neurotransmitter in the peripheral autonomic transmission. We report here the results of an investigation using dopaminoceptor agonists and antagonists on cholinergic transmission in the longitudinal muscle preparation of the guinea-pig ileum. Some aspects of a similar study have recently been reported by Ennis, Janssen, Schneiden & Cox (1979).

Plexus-containing longitudinal muscle strips were prepared according to Paton & Zar (1968) and were suspended in Krebs solution in 5 ml organ baths between platinum electrodes at 37°C and contractions were recorded isometrically. For electrical stimulation, single pulses (0.2 ms pulse duration, 0.1 Hz, supramaximal voltage) were used.

DA (10^{-6}–10^{-4} M) caused an inhibition of the electrically-evoked twitch (maximum % inhibition: 35.1 ± 3.0 s.e. mean). The threshold concentration of DA for inducing inhibition was around 10^{-6} M and maximum inhibition was usually achieved by 5×10^{-5} M of DA. The presynaptic nature of DA-induced inhibition of the electrically-evoked twitch was indicated by the finding that Ach-evoked contraction remained unaffected by DA. DA was approx. 1/100th as potent as noradrenaline in inhibiting the electrically-evoked twitch and in common with noradrenaline, DA-induced inhibition was effectively antagonised by phentolamine. Haloperidol (10^{-7} M) and pimozide (2×10^{-7} M) did not antagonise DA-induced inhibition of twitch. Higher concentrations of

haloperidol and pimozide reduced the electrically-induced twitches and Ach-evoked contractions without impairing the ability of DA to further-inhibit the twitch. Bromocriptine ($0.1–50 \times 10^{-6}$ M), a central DA-receptor agonist inhibited the electrically-evoked twitch and Ach-induced contraction. Unlike DA-induced inhibition, bromocriptine effect was not antagonised by phentolamine, nor was it counteracted by haloperidol, metoclopramide or pimozide.

DA $> 10^{-6}$ M, in the presence of phentolamine (5×10^{-6} M), potentiated the electrically-evoked twitch without a corresponding potentiation of Ach-induced contraction. Potentiation persisted unchanged after treatment with haloperidol (5×10^{-7} M) or indomethacin (3×10^{-6} g/ml). DA-induced potentiation was augmented after combined treatment with tyramine ($1–5 \times 10^{-4}$ M) and desmethylimipramine (10^{-6} M) but was antagonised by pimozide (2×10^{-6} M).

In conclusion, the above results indicate that DA is capable of exerting an effect upon the postganglionic cholinergic motor transmission in this preparation in two ways: first it can inhibit the transmission by activating the presynaptic α-adrenoceptors located on the cholinergic nerve terminals and secondly it can facilitate cholinergic transmission presumably by activating presynaptic specific dopaminoceptors. The physiological significance of these findings remains yet to be determined.

References

BJORKLUND, A., CEGRELL, L., FALCK, B., RITZEN, M. & ROSENGREN, E. (1970). Dopamine-containing cells in sympathetic gangli. *Acta physiol. scand.*, **78**, 334–338.

ENNIS, C., JANSEEN, P.A.J., SCHNEIDEN, H. & COX, B. (1979). Characterization of receptors on postganglionic cholinergic neurons in the guinea-pig isolated ileum. *J. Pharm. Pharmacol.* **31**, 217–221.

PATON, W.D.M. & ZAR, M.A. (1968). The origin of acetylcholine released from guinea-pig intestine and longitudinal muscle strips. *J. Physiol.* **194**, 13–33.

THORNER M.O. (1975) Dopamine—an important neurotransmitter in the autononic nervous system. *Lancet.* i, 662–664.

Spontaneous and evoked release of enkephalin from the myenteric plexus of guinea-pig small intestine

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We have reported that in the presence of cycloheximide to block *de nouveau* synthesis, the difference between the enkephalin contents of stimulated and unstimulated preparations of guinea-pig myenteric plexus-longitudinal muscle gives an indication of the amount of enkephalin released. Thus stimulation at 0.1 Hz produced no loss of enkephalin but at 1 and 10 Hz a maximal loss of about 50% of the tissue stores was obtained (Hughes, Kosterlitz and Sosa, 1978).

In the present study, a similar method was used to investigate the release of enkephalin using different current strengths at 10 Hz. Stimulated and control tissues (200–300 mg) were prepared from the one guinea-pig. During a 1 h preincubation in standard Krebs-bicarbonate solution, the contractions of the longitudinal muscle in response to stimulation of the cholinergic fibres at 0.1 Hz were monitored. Stimulation was started after a 30 min equilibration period in Krebs containing cycloheximide (0.1 mM) and a mixture of amino acids (Hughes *et al.*, 1978). The enkephalin contents were measured by bioassay on the mouse vas deferens after extraction of the tissues in 0.1 M HCl and adsorption onto Amberlite XAD-2 (Hughes, Kosterlitz & Smith, 1977).

The depleting effect of stimulation increased with time. A maximal depletion of approximately 50% was seen after 1–2 h with 75 or 100 mA but with 50 mA, 3–4 h stimulation was required to produce this effect. At 50 mA the fractional release per pulse was constant for up to 4 h at approx. 0.5×10^{-5}. At 75 and 100 mA the fractional releases decreased from approx. 2×10^{-5} after 30 min to 0.5×10^{-5} after 2 hours. After 2 h the absolute amount of enkephalin released was at a minimum; approx. 2 fmol/g/pulse with all currents. At 30 min the amount released with 50 mA was only 4 fmol/g/pulse while with 75 and 100 mA the amount was 10–12 fmol/g/pulse. Thus for the release of enkephalin 50 mA appears to be a submaximal stimulus although in the same preparation 50 mA was a maximal stimulus for release of acetylcholine. Therefore the enkephalinergic fibres in the myenteric plexus may be different from the cholinergic fibres and possibly of a narrower diameter.

The possibility that there might have been spontaneous release of enkephalin was considered, especially since the amount of enkephalin released per pulse decreased with time at a somewhat greater rate than the fractional release. Regression analysis of the unstimulated, control data revealed a positive correlation ($r = 0.646$, d.f. $= 30$, $P < 0.001$) suggesting that

the contents fell by approx. 50% over 4 hours. In a few preliminary experiments, the contents of tissues incubated for 4 h with tetrodotoxin (3 μM) did not vary significantly from the contents of control tissues. We have concluded that although there may be a spontaneous loss of enkephalin from the myenteric plexus of guinea-pig small intestine, this is not due to release associated with nerve activity.

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References

HUGHES, J., KOSTERLITZ, H.W. & SMITH, T.W. (1977). The distribution of methionine-enkephalin and leucine-enkephalin in the brain and peripheral tissues. *Br. J. Pharmac.*, **61**, 639–647.

HUGHES, J., KOSTERLITZ, H.W. & SOSA, R.P. (1978) Enkephalin release from the myenteric plexus of the guinea-pig small intestine in the presence of cycloheximide. *Br. J. Pharmac.*, **63**, 397P.

Histamine H_1- and H_2-receptor mediated effects in vasa deferentia from the rat, guinea-pig and rabbit

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Histamine inhibits the electrically-induced twitch response of the mouse vas deferens via a histamine H_2-receptor (Marshall, 1978; Bhalla & Marshall, 1979). The effect of histamine on isolated vasa deferentia from the rat, guinea-pig and rabbit has been investigated.

Vasa deferentia from rats (250–350 g), guinea-pigs (350–450 g) and rabbits (2.5–3.0 kg) were bisected and each portion suspended in oxygenated magnesium-free Krebs solution at 37°C between parallel platinum electrodes. Responses to stimulation (0.2 Hz, 2.0 ms, 64 V; Grass S44 Stimulator) were recorded isometrically.

Histamine (0.1 μM–3.0 mM) inhibited the twitch response of the prostatic portion of the rat vas deferens in a concentration-dependent manner (IC_{50}, concentration to inhibit the twitch by 50%, 222 ± 44 μM, mean ± s.e. mean). The maximum inhibition obtained varied between 95 and 100%. The histamine H_2-receptor antagonist cimetidine (30–300 μM) shifted the concentration-effect curves to histamine in parallel to the right (dose ratios, DR; 6.9 ± 0.2, 10.9 ± 1.3 and 16.2 ± 4.0 for cimetidine 30, 100 and 300 μM respectively). The histamine H_1-receptor antagonist mepyramine (1.0 μM) was ineffective against the histamine inhibition. The inhibitory action of histamine was mimicked by the selective histamine H_2-receptor agonist dimaprit (IC_{50}, 942 ± 160 μM), and this was also antagonised by cimetidine (100 μM) (DR 9.8 ± 2.4).

The twitch response of the epididymal portion of the rat vas deferens was inhibited by low concentrations of histamine (0.1–300 μM) but potentiated by higher concentrations (1.0–10 mM). The maximum inhibition obtained was 55 ± 1.2% and this was increased in the presence of mepyramine (1.0 μM) to 74 ± 2.2%. Cimetidine (100 and 300 μM) antagonised the inhibitory action of histamine but was ineffective against the twitch potentiation. Mepyramine (1.0 μM) antagonised the histamine-induced potentiation, but not the inhibition. Dimaprit (3.0 μM–3.0 mM) also inhibited the twitch response of the epididymal end of the rat vas deferens (IC_{50}, 160 and 260 μM, 2 experiments) with a maximum inhibition of 95% and 98%. Cimetidine (300 μM) antagonised the action of dimaprit (DR, 14.5 and 17.9, 2 experiments).

Twitch responses of the epididymal and prostatic portions of the vasa deferentia of the guinea-pig and rabbit were unaffected by dimaprit (0.1 μM–1.0 mM) and potentiated by histamine (1.0 μM–1.0 mM). The potentiation was antagonised by mepyramine (1.0 μM) but not by cimetidine (300 μM). The two portions of the guinea-pig vas deferens were contracted by histamine (10–300 μM) in the absence of stimulation. This action was abolished by mepyramine (100 nM) while cimetidine (300 μM) was ineffective.

In conclusion, the histamine inhibition of the twitch response of the isolated vas deferens of the rat, like that of the mouse, is mediated via a histamine H_2-receptor. Conversely, in vasa from the rat (epididymal portion), guinea-pig and rabbit, the potentiation of the twitch produced by histamine, together with the histamine-induced contraction of the guinea-

pig vas deferens appear to be mediated via histamine H_1-receptors.

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References

BHALLA, P. & MARSHALL, I. (1979). Histamine H_2-receptor antagonists in the mouse isolated vas deferens, *Br. J. Pharmac.* (B.P.S. Leeds, 1979).

MARSHALL, I. (1978). An inhibitory histamine H_2-receptor in the mouse vas deferens. *Br. J. Pharmac.*, **62**, 447P.

Histamine receptors in guinea-pig airways

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The smooth muscle of airways is contracted (Arunlakshana & Schild, 1959) and relaxed (Eyre, 1973) by histamine acting on H_1 and H_2 receptors respectively. We have observed that cimetidine and metiamide potentiate and impromidine (H_1 antagonist and H_2 agonist; Durant, Duncan, Ganellin, Parsons, Blakemore and Rasmussen, 1978) inhibits histamine-induced bronchoconstriction and death in guinea-pigs. The H_2 receptor antagonists do not block the effect of impromidine. It would appear therefore, that cimetidine, metiamide and impromidine are not all acting on H_2 receptors. This suggestion is supported by the observation that dimaprit, an H_2 agonist without H_1 antagonist properties (Parsons, Owen, Ganellin and Durant, 1977) does not prevent histamine induced bronchoconstriction and death in guinea-pigs. Impromidine and the H_2 antagonists may be acting at another type of receptor. An attempt has been made to elucidate the site and mechanism of action of H_2 antagonists and impromidine on histamine induced constriction of guinea-pig airways *in vitro*.

Spirally cut strips of trachea and bronchus and parenchymal strips of the lung (Drazen and Schneider, 1978) were equilibrated in Krebs' solution at 37°C and gassed with 95% O_2 5% CO_2 for 0.5–1 hour at resting tensions of 2 g, 1 g, and 200 mg respectively. Two point dose response curves were obtained in response to histamine, 2-methylhistamine and barium chloride three times, before and after addition of the drugs under test. Each experiment was repeated four times. Changes in tension were recorded isometrically and were calculated as percentages of the response to acetylcholine (5×10^{-4} M). Mepyramine (pA_2 trachea 9.83, bronchus 9.11, parenchymal strip 9.11) and impromidine (pA_2 trachea 5.15, bronchus 4.71, parenchymal strip 5.37) inhibited histamine-induced contractions of each preparation. Dimaprit (10^{-4} M) reduced the contractions of the trachea but not those of the smaller airways to histamine ($10^{-5.7}$–10^{-5} M). Cimetidine (10^{-4} M) potentiated the response of the parenchyma to histamine but not those to either 2-methylhistamine or barium chloride. Metiamide (10^{-4} M) also potentiated histamine induced contractions of the parenchymal strip. Cimetidine had a similar but smaller effect on the trachea but no effect on the contractions of the bronchus induced by histamine. Furthermore, cimetidine (10^{-4} M) did not reverse the impromidine (10^{-4} M) inhibition of the actions of histamine on the parenchymal strips.

These observations suggest that in previous experiments, the site of action of impromidine is in both the large and small airways whilst that of the H_2 antagonists is confined mainly to the small airways. The results are consistent with the view that impromidine produces its effects by antagonising the H_1 actions of histamine in respiratory smooth muscle. It seems unlikely that impromidine is acting at H_2 receptors because the pA_2 values for either mepyramine or impromidine are not significantly different for each tissue and are similar to those for guinea-pig ileum (Schild, 1947; Durant *et al.*, 1978). Dimaprit has no effect in the parenchymal tissue (where the effects of cimetidine and metiamide are observed) and cimetidine does not modify the effects of impromidine. However, these facts do not exclude the possibility that cimetidine and metiamide are acting on H_2 receptors as dimaprit might not be expected to further stimulate H_2 receptors in the presence of histamine. An alternative explanation is that the effects of the H_2 antagonists are due to a reduction in the uptake or breakdown of histamine (Taylor, 1973; Fantozzi, Franconi, Mannaioni, Masini, Maroni, 1975).

References

ARUNLAKSHANA, O. & SCHILD, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmac.*, **14**, 48–58.

DRAZEN, J.M. & SCHNEIDER, M.W. (1978). Comparative responses of tracheal spirals and parenchymal strips to histamine and carbachol *in vitro*. *J. Clin. Invest.*, **61**, 1441–1447.

DURANT, G.J., DUNCAN, W.A.M., GANELLIN, C.R., PARSONS, M.E., BLAKEMORE, R.C. & RASMUSSEN A.C. (1978). Impromidine (SK & F 92676) is a very potent and specific agonist for histamine H_2 receptors. *Nature*, **276**, 403–404.

EYRE, P. (1973). Histamine H_2 receptors in the sheep bronchus and cat trachea: the action of burimamide. *Br. J. Pharmac.*, **48**, 321–323.

FANTOZZI, R., FRANCONI, F., MANNAIONI, P.F., MASINI, E., & MARONI, F. (1975). Interaction of histamine H_1 and H_2 receptor antagonists with histamine uptake and metabolism by guinea-pig isolated atrium and mouse neoplastic mast cells *in vitro*. *Br. J. Pharmac.* (1975), **53**, 569–574.

PARSONS, M.E., OWEN, D.A.A., GANELLIN, C.R., & DURANT, G.J. (1977). Dimaprit [S-[3-(N,N-dimethylamino)propyl]isothiourea] a highly specific histamine H_2 receptor agonist. *Agents and Actions*, **7**, 31–37.

SCHILD, H.O. (1947). pA, a new scale for the measurement of drug antagonism. *Br. J. Pharmac.*, **2**, 189–206.

TAYLOR, K.M. (1973). Comparison of the antihistamine effect of burimamide and mepyramine and their effect on the activity of histamine methyltransferase. *Biochem. Pharmac*, **22**, 2775–2776.

Anti-bronchoconstrictor activity of selected phosphodiesterase inhibitors and their effects on lung cyclic nucleotides

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The finding that the smooth-muscle-relaxing drugs, theophylline and papaverine were also inhibitors of phosphodiesterase led to the suggestion that their therapeutic properties in the treatment of reversible airways disease (bronchoconstriction) were related to their ability to increase the levels of cyclic nucleotides in the lung (Kukovetz & Poch, 1970). This investigation was performed to compare the effects of four phosphodiesterase inhibitors, theophylline, papaverine, ICI 63197 and isobutylmethylxanthine (IMX) on histamine-induced bronchoconstriction and on cyclic nucleotide levels in guinea-pig isolated lung lobes.

Single lung lobes were perfused as previously described (Beswick, Davies & Goadby, 1977) and the perfusion pressure measured by pressure transducers. Bronchoconstriction caused by histamine (100 ng–100 μg) was manifested as an increase in the perfusion pressure. Dose-response curves to histamine were performed before and after perfusion of the lobes with the phosphodiesterase inhibitors (1–1000 μg/ml).

Table 1 Levels of cyclic nucleotides (p mol/mg protein) in isolated, perfused guinea-pig lung lobes after treatment with histamine (50 μg) and selected phosphodiesterase inhibitors.

		Cyclic AMP		*Cyclic GMP*	
Drug	*Concentration*	*Control*	*Histamine*	*Control*	*Histamine*
		8.7 ± 0.48	14.2 ± 0.44**	0.66 ± 0.14	1.10 ± 0.09**
Papaverine	10 μg/ml	9.6 ± 0.27	10.1 ± 0.80	0.60 ± 0.04	0.60 ± 0.05
	100 μg/ml	13.3 ± 1.40*	13.1 ± 1.87	1.20 ± 0.10*	1.20 ± 0.10
ICI 63197	10 μg/ml	9.1 ± 0.89	10.7 ± 1.30	0.80 ± 0.26	0.79 ± 0.14
	100 μg/ml	13.5 ± 0.90*	13.2 ± 1.30	0.90 ± 0.25*	0.85 ± 0.10
IMX	10 μg/ml	7.6 ± 0.40	8.0 ± 0.40	0.60 ± 0.20	0.60 ± 0.09
	100 μg/ml	12.4 ± 2.10*	12.6 ± 1.60	0.80 ± 0.10*	0.78 ± 0.10
Theophylline	100 μg/ml	8.7 ± 0.19	12.5 ± 0.30**	0.80 ± 0.02	1.14 ± 0.05**
	1 mg/ml	14.8 ± 1.60*	14.3 ± 1.60	1.10 ± 0.09*	1.17 ± 0.07

Values are the mean ± s.e. mean of 6 observations.
* Significant ($P \leq 0.05$) elevations over basal nucleotide levels.
** Significant ($P \leq 0.05$) elevations of nucleotide levels after histamine treatment.

All four compounds caused a dose-dependent inhibition of histamine-induced bronchoconstriction in the lobes. This inhibition was non-competitive in nature. IMX was the most potent inhibitor, followed by papaverine and ICI 63197, the dose ratios at a concentration of 1 μg/ml being 20, 15 and 10 respectively. Theophylline was the least effective of the four, only producing a marked inhibition when the concentration was raised to 1 mg/ml.

Using the same perfusion system, the effect of the four compounds on the levels of cyclic AMP and cyclic GMP in the lung lobes was investigated. Nucleotide levels were measured in untreated lung lobes and in lobes which had been constricted with histamine (50 μg). At the height of the response the lung tissue was snap frozen in liquid air, ground to a powder and extracted with trichloroacetic acid (0.4 N) as described by Steiner, Pagliara, Chase & Kipnis (1972). The nucleotides were estimated by means of the Radiochemical Centre (Amersham) cyclic AMP and cyclic GMP assay kits.

The results (Table 1) show that histamine constriction was accompanied by a rise in the levels of both nucleotides. However, although the phosphodiesterase inhibitors caused increases in cyclic nucleotide levels, there was no further increase on addition of histamine except in the case of theophylline (100 μg/ml) but this dose was ineffective in inhibiting the bronchoconstriction. These results suggest that the antibronchoconstrictor activity is related to the ability to prevent the increase in cyclic nucleotides caused by histamine rather than an effect on basal levels of these nucleotides.

References

BESWICK, S.P., DAVIES, J.S. & GOADBY, P. (1977). Regional perfusion of the airways of guinea-pig lung: a selective action of histamine on the smooth muscle of the small airways. *Br. J. Pharmac.*, **61**, 156P.

KUKOVETZ, W.R. & POCH, F. (1970). Inhibition of cyclic-3′,5′-nucleotide-phosphodiesterase as a possible mode of action of papaverine and similarly acting drugs. *Naunyn-Schmiedebergs Arch. Pharmak.*, **267**, 189–194.

STEINER, A.L., PAGLIARA, A.S., CHASE, L.R. & KIPNIS, D.M. (1972). Radio-immunoassay for cyclic nucleotides. II. Adenosine 3′,5′-monophosphate in mammalian tissues and body fluids. *J. Biol. Chem.*, **247**, 1114–1120.

Inhibition of Forssman-induced bronchospasm in the guinea-pig

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Forssman shock is an acute lethal syndrome provoked in guinea-pigs by i.v. injection of rabbit antiserum to sheep erythrocyte stroma. The antibodies contained therein cross-react with antigens situated on or near the vascular endothelium (Tanaka & Leduc, 1956). Both complement (May & Frank, 1972) and platelets (Taichman & Tsai, 1975) are essential for the pathogenesis of the reaction. Increasing doses of antisera result in an increasing severity of thrombocytopenia and platelet sequestration in the pulmonary vasculature (Butler & White, 1979).

Bronchospasm was measured using the method of Mathe, Strandberg & Fredholm (1972) and all drugs were administered into the jugular vein. A low dose (sublethal) of antiserum (0.2 ml) was used to induce a biphasic bronchospasm and subsequent drug studies were carried out using this dose of antiserum.

Both phase I and II were dependent on an intact complement system and circulating platelets since pretreatment with cobra venom factor (200 units/kg), neuraminidase (2 units/kg) or antiplatelet antiserum (0.5 ml/kg) for 3 days prior to the experiment abolished both phases of bronchospasm.

Phase I bronchospasm appeared to be mediated by serotonin release since it was selectively blocked by methysergide (0.3 mg/kg). This phase was also blocked by aspirin (100 mg/kg), indomethacin (10 mg/kg) and sulphinpyrazone (100 mg/kg), probably as a result of inhibition of the platelet release reaction. These doses of the non-steroidal anti-inflammatories also blocked phase II bronchospasm but only sulphinpyrazone prevents the thrombocytopenia at this stage (Butler & White, 1979). Sulphinpyrazone significantly inhibited phase II at doses down to 30 mg/kg. Inhibition of this phase of the reaction might be due to a general inhibition of prostaglandin synthetase activity rather than a direct platelet effect, however, since sulphinpyrazone appears more potent than aspirin at this stage other factors might also be important. Mepyramine also blocked phases I and II but since Humphrey & Mota (1959) demonstrated no mast cell degranulation by anti-Forssman antibody

its antagonism was probably not directed against a histamine component.

Aspirin (10 mg/kg), sodium salicylate (30 mg/kg) and indomethacin (1 mg/kg) given 10 min prior to sulphinpyrazone (30 mg/kg) were all capable of inhibiting the action of sulphinpyrazone on phase II bronchospasm. This action might be mediated via the cyclooxygenase system in the same fashion as Vargaftig (1978) demonstrated for the prevention of aspirin-induced inhibition of the platelet cyclooxygenase by salicylic acid.

References

BUTLER, K.D. & WHITE, A.M. (1979). Inhibition of the platelet involvement in the sublethal Forssman reaction by sulphinpyrazone and not by aspirin. In '*Anturane*' International symposium, Bern Stuttgart Vienna (Hans Huber) (in press).

HUMPHREY, J.H. & MOTA, I. (1959). The mechanism of anaphylaxis: specificity of antigen induced mast cell damage in anaphylaxis in the guinea pig. *Immunology*, **2**, 31–43.

MATTHE, A.A., STRANDBERG, K. & FREDHOLM, B. (1972). Antagonism of prostaglandin $F_{2\alpha}$ induced bronchospasm. *J. Pharm. Pharmac.*, **24**, 378–382.

MAY, J.E. & FRANK, M.M. (1972). Complement mediated tissue damage: contribution of the classical and alternate pathways in the Forssman reaction. *J. Immunol.*, **108**, 1517–1525.

TAICHMAN, N.S. & TSAI, C.C. (1975). Platelets, Drugs and Intravascular Immune Reactions. In '*Platelets, Drugs and Thrombosis*' Symposium, Hamilton 1972 pp. 169–181. Eds. Hirsh, J., Cade, J.F., Gallus, A.S. and Schönbaum, E. (Karger-Basle).

TANAKA, N. & LEDUC, E.H. (1956). A study of the cellular distribution of Forssman antigen in various species. *J. Immunol.*, **77**, 198–212.

VARGAFTIG, B.B. (1978). The inhibition of cyclooxygenase of rabbit platelets by aspirin is prevented by salicylic acid and by phenanthrolines. *Eur. J. Pharmacol.*, **50**, 231–241.

The effect of endotoxin shock on histamine and histidine decarboxylase enzyme activities in various tissues of cats

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The present study is an attempt to correlate early cardiovascular changes following endotoxin administration and blood and tissue levels of histamine and histamine-forming enzymes in cats. *E. coli* endotoxin (Difco Laboratories, 055:B5) in a dose of 2 mg/kg suspended in saline, was injected intravenously over a period of 40–45 seconds. Blood pressure and blood histamine were determined at one, three, five and 10 minutes. There was a marked and progressive decrease in systemic arterial pressure reaching as low as 58 mmHg (normal 120 ± 6 mm Hg) within three minutes. This was followed by a partial recovery after five and 10 minutes.

In the plasma and cells, histamine did not show a distinct change. At five and 10 min after endotoxin, cats were sacrificed, and the histamine content, specific and non-specific histidine decarboxylase enzyme activities were determined in the various tissues (Waton, 1956, 1964). The early phase of endotoxin shock was associated with an increase in specific histidine decarboxylase activity in the skin, intestine, lung and stomach. A less pronounced change was observed in the liver, heart and kidneys. There was also a highly significant rise in the concentration of histamine in the lung, intestine, skin and liver.

The data presented add further support to the concept that newly synthesized histamine plays in the pathophysiology of shock and that endotoxin is a potent activator of histidine decarboxylase.

References

WATON, N.G. (1956). Studies on mammalian histidine decarboxylase. *Br. J. Pharmac. and Chemother.*, **11**, 119–127.

WATON, N.G. (1964). Uptake and formation of histidine by cat tissues. *J. Physiol., Lond.*, **172**, 475–481.

Effect of $PGF_{2\alpha}$, PGE_2 and ICI 81008 on the *in vivo* and *in vitro* uterus of non-pregnant rats and guinea-pigs

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Prostaglandins (PG's) of the E and F series stimulate the non-pregnant uterus of most animals both *in vitro* and *in vivo* (Bergström, Carlson & Weeks, 1968). The rank order of potency of these PG's has been shown to be different on the longitudinal uterine smooth muscle of the rat (F's > E's) and guinea-pig (E's > F's), (Whalley & White, 1979). This study compares the effect of $PGF_{2\alpha}$, PGE_2 and the $PGF_{2\alpha}$ analogue ICI 81008, a compound which has previously been described as a relatively selective luteolytic agent (Dukes, Russel & Walpole, 1974), on the *in vivo* and *in vitro* longitudinal and circular uterine smooth muscle of the non-pregnant rat and guinea-pig.

Uterine activity was recorded *in vivo* in conscious rats and guinea-pigs as described previously (Whalley & Riley, 1979). PG's were injected intra-arterially. The rank order of potency of the PG's on the *in vivo* uterus of both rats and guinea-pigs was ICI 81008 > $F_{2\alpha}$ > E_2. PGE_2 often produced bell-shaped dose-response curves in both species and in guinea-pigs dose-related inhibition of spontaneous uterine activity was sometimes observed. Since *in vivo* uterine activity recorded by a microballoon may reflect circular muscle activity, the action of the PG's were compared on the *in vitro* longitudinal and circular uterine smooth muscle of rats and guinea-pigs using the method described previously (Whalley & White, 1979). The relative potencies of the PG's were determined by comparing the mean (n = 7–29) Molar EC_{50} of each PG to that of $PGF_{2\alpha}$ (assigned a potency = 1). The results are shown in Table 1. On the rat uterus, the threshold concentration of each PG was similar on the longitudinal and circular muscle whilst that of the guinea-pig was greater on the circular compared to longitudinal muscle. In addition, the maximal responses produced by the guinea-pig circular muscle were significantly reduced compared to the longitudinal muscle. These results demonstrate marked differences in the reactivity of the *in vivo* and *in vitro* uterus of the rat and guinea-pig to $PGF_{2\alpha}$, PGE_2 and ICI 81008.

Table 1 Relative potencies of $PGF_{2\alpha}$ (assigned a potency = 1), PGE_2 and ICI 81008 in stimulating contractions of *in vitro* longitudinal (L) and circular (C) uterine smooth muscle of non-pregnant rats and guinea-pigs.

	Rat uterus		*Guinea-pig uterus*	
Compound	*L*	*C*	*L*	*C*
$PGF_{2\alpha}$	1	1	1	1
PGE_2	0.3	0.2	20.3	33
ICI 81008	20.3	16.5	0.01	†

† not determined.

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References

BERGSTRÖM, S., CARLSON, L.A. & WEEKS, J.R. (1968). The prostaglandins: a family of biologically active lipids. *Pharmac. Rev.*, **20**, 1–48.

DUKES, M. & RUSSELL, W. & WALPOLE, A.L. (1974). Potent luteolytic agents related to prostaglandin $F_{2\alpha}$. *Nature, Lond.*, **250**, 330–331.

WHALLEY, E.T. & RILEY, A.J. (1979). The effect of aprotinin on uterine contractile mechanisms in the pregnant rat at term. *J. Rep. Fert.*, **55**, 377–384.

WHALLEY, E.T. & WHITE, S.K. (1979). Comparison of various prostaglandins (PG's) on the *in vitro* longitudinal uterine smooth muscle of the rat and guinea-pig. *Br. J. Pharmac.* (In press).

Species variations in the kinetics of SL 75 212, a new β-adrenoceptor antagonist

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The kinetics and metabolic profile of SL 75 212, (±)-1-isopropylamino-3-[4-(2-cyclopropylmethoxyethyl)phenoxyl]propan-2-ol.HCl, have been examined in rat, dog and man during a search for an animal species with a drug-handling ability similar to man.

After oral administration of a tracer dose of the compound labelled with ^{14}C in the C1 of the cyclopropylmethoxy side chain (280 μg, 50 μCi) to a human volunteer, 66% of the ^{14}C was recovered in the 0–48 h urine. Male rats excreted 88.2 ± 1.65% of an oral dose (1 mg/kg, 100 μCi/kg) and male beagle dogs (1 mg/kg, 15 μCi/kg) 60.7 ± 4.0% in urine over the same time period. The remainder of the dose was excreted in the faeces in all three species. In rat and dog, similar urinary excretion values were found after either oral or intravenous dosing indicating that the drug is well absorbed.

In contrast to the similar urinary excretion total in the three species, the plasma clearance kinetics of rat, dog and man were found to be significantly different. Thus, $T_{\frac{1}{2}}\ \beta$ for total radioactivity in the plasma after oral dosing was 2.25 ± 0.25 h in the rat, 33.4 ± 1.8 h in the dog and 16.4 h in man. The $T_{\frac{1}{2}}\ \beta$ for the unchanged drug was found to be 2.45 ± 0.5 h in the rat, 3.95 ± 0.09 h in the dog compared with 14.3 ± 0.7 h in man, as reported by Bianchetti, Gomeni, Kilborn, Morselli, Taylor & Warrington (1979).

The AUC $_{0 \rightarrow \infty}$ for unchanged drug was 82 ± 14 (i.v.) and 13 ± 3 (p.o.) in the rat and 841 ± 48 (i.v.) and 641 ± 54 (p.o.) (units, μg L^{-1} h) in the dog. This indicates that there is a marked 'first pass' effect in the rat but that this is barely perceptible in the dog.

SL 75 212 is well metabolized by all three species since only 6.4% (pooled 0–48 h) was recovered unchanged in the urine of rat, 5.6 ± 0.7% (0–48 h) in dog and 12 ± 2.5% in man (Bianchetti *et al.*, 1979).

Interestingly the plasma half lives for the closely related compound metoprolol are in the same ranking order, but are much shorter (rat 0.62 ± 0.07 h, dog 1.61 h, Borg, Fellenius, Johansson & Wallborg, 1975, and man 3.3 ± 0.1 h, Kendall, Brown, Grieve & John, 1977) and this is in all probability due to the absence of the cyclopropyl group which is present in SL 75 212.

References

BIANCHETTI, G., GOMENI, R., KILBORN, J.R., MORSELLI, P.L., TAYLOR, E.A. & WARRINGTON, S.J. (1979). Blood concentrations and pharmacodynamic effects of SL 75 212, a new β-adrenoceptor antagonist, after oral and intravenous administration. *Brit. J. clin. Pharmacol.* **8**, 403–404.

BORG, K.O., FELLENIUS, E., JOHANSSON, R. & WALLBORG, M. (1975). Pharmacokinetic studies of metoprolol-(^3H) in the rat and the dog. *Acta pharmacol. toxicol.*, **36**, suppl. V, 104–115.

KENDALL, M.J., BROWN, D., GRIEVE, A. & JOHN, V.A. (1977). Pharmacokinetic and pharmacodynamic studies of single oral doses of metoprolol in normal volunteers. *Eur. J. Drug Metab. Pharmacokin.*, **2**, 73–80.

Pharmacokinetics of vitamin K_1 in young and aged rats

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A previous study has shown that elderly human subjects and rats show an increased sensitivity to warfarin (Shepherd, Hewick, Moreland & Stevenson, 1977) which is apparently not attributable to changes in warfarin pharmacokinetics. Relative to the plasma warfarin concentration, there was in the elderly of both species a greater inhibition of vitamin K_1-dependent prothrombin complex synthesis. In an attempt to explain this, the possibility that the pharmacokinetics of vitamin K_1 may be altered in aged rats has been investigated.

Young and aged rats (male, Sprague-Dawley, 90 and 477 days respectively) received a single oral dose of [^3H]-vitamin K_1 (10 μg/kg, 7.5 μCi/kg) and were sacrificed at intervals up to 9 h after dosing. In a separate study, following the same dose of [^3H]-vitamin K_1 given intravenously, the biliary excretion of vitamin K_1 was investigated in urethane anaesthetised, bile duct-cannulated rats.

Plasma and liver samples were analysed for vitamin K_1 content and total lipid-soluble radioactivity re-

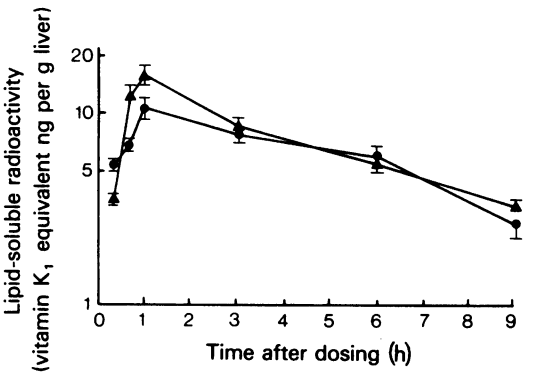

Figure 1 Liver lipid-soluble radioactivity after single oral dosing of [^{3}H]-vitamin K_1 (10 μg/kg, 7.5 μCi/kg) in young (▲) and old (●) rats (three rats per data point: mean ± s.e. mean).

spectively (Shearer, Barkhan & Webster, 1970). Spleen, heart and kidney were also removed for estimation of total radioactivity.

Liver radioactivity was accounted for almost entirely in terms of a lipid-soluble fraction. Figure 1 shows for both age groups the liver lipid-soluble radioactivity versus time profile. The mean half-life ($T_{\frac{1}{2}}$) values obtained for this were 3.7 h (young) and 4.0 h (old) and did not differ significantly. Although the mean vitamin K_1 plasma $T_{\frac{1}{2}}$ value in the elderly group was greater (5.2 h compared with 2.8 h), again the difference was not significant. The total concentration of radioactivity was greatest in liver followed by plasma, spleen, kidney and heart respectively. The total radioactivity levels at 1 h after dosing were generally lower in tissues from the elderly rats but at 3 h there were no significant differences between the age groups. In the bile study no difference was found between the groups in total radioactivity excreted in 6 h, the radioactivity being mainly unidentified metabolites.

This investigation indicates that there is no gross age-related differences in vitamin K_1 handling in non-anticoagulated animals. These results are in agreement with a previous study in both anticoagulated and non-anticoagulated patients (Shepherd, Wilson & Stevenson, 1979).

We are grateful to Professor J.G. Phillips, Wolfson Research Laboratory in Gerontology, University of Hull, for the supply of aged animals and to Dr. R.F. Long, Roche Products Ltd., Welwyn Garden City, Herts. for [^{3}H]-vitamin K_1.

References

SHEARER, M.J., BARKHAN, P. & WEBSTER, G.R. (1970). Absorption and excretion of an oral dose of tritiated vitamin K_1 in man. *Brit. J. Haemat.* **18**, 297–308.

SHEPHERD, A.M.M., HEWICK, D.S., MORELAND, T.A. & STEVENSON, I.H. (1977). Age as a determinant of sensitivity to warfarin. *Brit. J. Clin. Pharmac.* **4**, 315–320.

SHEPHERD, A.M.M., WILSON, N.M. & STEVENSON, I.H. (1979). Warfarin sensitivity in the elderly. In *Drugs and the Elderly*, ed. Crooks, J. & Stevenson, I.H. pp. 199–209, London: Macmillan Press.

Ophthalmic effects of intramuscularly administered pralidoxime mesylate

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Oximes, including pralidoxime mesylate (P2S; 2-hydroxyiminomethyl-N-methylpryridinium methanesulphonate), are therapeutically and prophylactically effective in organophosphorus anticholinesterase poisoning. Normal human volunteer subjects given oximes for pharmacokinetic studies have experienced visual disturbances, including diplopia and difficulties with accommodation, which could adversely affect performance in conditions requiring oxime prophylaxis (Holland & Parkes, 1976; Sundwall, 1960). Such effects could be mediated centrally or by a direct action on the eye.

In preliminary animal studies, P2S was detected in aqueous humor following large, potentially toxic, systemic injections of P2S (Ballantyne, Gazzard, Robson & Swanston, 1975). These studies have been extended by measuring intraocular tension (IOT) and aqueous humor and plasma P2S concentrations following i.m. injection of P2S (10, 40 and 100 mg/kg) to adult female Porton-strain rabbits (six for each treatment group).

Sequential measurements of IOT by applanation tonometry (Ballantyne, Gazzard & Swanston, 1977) demonstrated peak increases of 7.3% (10 mg/kg), 7.6% (40 mg/kg), and 12.4% (100 mg/kg) at 30 minutes. In a separate experiment, IOT was measured at various times after dosing and the animals sacrificed after IOT measurement for removal of blood and aqueous humor for P2S determinations (Creasey & Green, 1959). Results are shown in Figure 1. P2S was detected in aqueous humor following 100 and 40 mg/kg, but not 10 mg/kg. Plasma P2S concentrations were above the experimentally determined therapeutic level of 4 μg/ml (Sundwall, 1961) for 45 min with 10 mg/kg, 2 h with 40 mg/kg, and 3 h with 100 mg/kg. Increases in IOT and the concentration duration curves for both aqueous humor and plasma P2S were dose-dependent. However, there was no statistical correlation between plasma or aqueous humor P2S and increases in IOT, or between plasma and aqueous humor P2S concentrations.

The results demonstrate that P2S enters aqueous humor following its parenteral administration, and is associated with changes in intraocular fluid dynamics. Thus, some ophthalmic side-effects of oximes could result from local actions on the eye. However, the absence of P2S in aqueous humor at 10 mg/kg suggests that other mechanisms may also be involved.

References

BALLANTYNE, B., GAZZARD, M.F., ROBSON, D.C. & SWANSTON, D.W. (1975). Concentrations of 2-hydroxyiminomethyl-N-methylpyridinium ion in plasma and aqueous humor as indices of the toxicity of pralidoxime mesylate (P2S) for the rabbit. *Toxicol. Appl. Pharmac.*, **33**, 559–567.

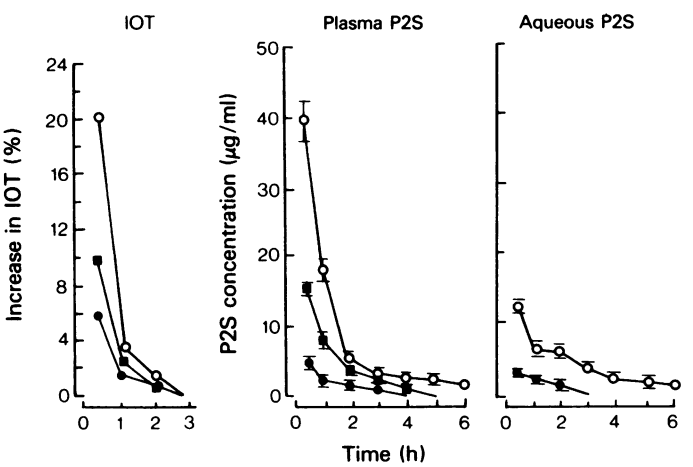

Figure 1 Increases in intraocular tension (IOT, left), plasma P2S concentrations (centre) and aqueous humor P2S concentrations (right) following i.m. P2S at doses of 100 mg/kg (○), 40 mg/kg (■) and 10 mg/kg (●). IOT was measured in various groups at the particular times shown and the animals then sacrificed for removal of blood and aqueous humor. Each point represents the mean ± s.e. mean for six rabbits.

BALLANTYNE, B., GAZZARD, M.F. & SWANSTON, D.W. (1977). Applanation tonometry in ophthalmic toxicology. In *Current Approaches in Toxicology*. ed. Ballantyne, B., pp. 158–192 Bristol, Wright.

CREASEY, N.H. & GREEN, A.L. (1959). 2-Hydroxyiminomethyl-N-methylpyridinium methanesulphonate (P2S), as an antidote to organophosphorus poisoning. Its preparation, estimation, and stability. *J. Pharm. Pharmac.*, **11**, 485–490.

HOLLAND, P. & PARKES, D.C. (1976). Plasma concentrations of the oxime pralidoxime mesylate (P2S) after repeated oral and intramuscular administration. *Brit. J. Industr. Med.*, **33**, 43–46.

SUNDWALL, A. (1960). Plasma concentration curves of N-methyl-pyridinium-2-aldoxime methanesulphonate (P2S) after intravenous, intramuscular, and oral administration in man. *Biochem. Pharmac.*, **5**, 225–230.

SUNDWALL, A. (1961). Minimum concentrations of N-methylpyridinium-2-aldoxime methanesulphonate (P2S) which reverse neuromuscular block. *Biochem. Pharmac.*, **8**, 413–417.

Does reactivation of phosphorylated acetylcholinesterase (AChE) in the brain enhance the antidotal actions of pyridinium aldoximes?

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Pyridinium aldoximes have a low lipid solubility, and it is debatable whether their antidotal action against lipid soluble anticholinesterases of the organo-phosphate type is associated with any reactivation of phosphorylated AChE in the brain (Hobbiger & Vojvodić, 1966). Lipid soluble oximes such as mono*iso*nitrosoacetone, on the other hand, also reactivate phosphorylated AChE in the brain, and judging by the reactivating potency determined *in vitro*, are more effective antidotes than are pyridinium aldoximes (Rutland, 1958). It is tempting, therefore, to conclude that the antidotal action of pyridinium aldoximes might be enhanced if a lipid soluble pro-drug could be synthesised which after penetration into the brain was converted into a pyridinium aldoxime. This was accomplished by Bodor, Shek & Higuchi (1976) who synthesised N-methyl-1,6-dihydropyridine-2-carbaldoxime HCl (pro-PAM) which is lipid soluble and after penetration into the brain is converted into N-methylpyridinium-2-carbaldoxime chloride (PAM). The same authors also showed that in mice treated with dyflos (di*iso*propylfluorophosphate) pro-PAM produced a much greater reactivation of the phosphorylated AChE in the brain than did PAM, given in equimolar doses (Shek, Higuchi & Bodor, 1976). In a detailed study Clement (1978) then observed that although pro-PAM, given prophylactically alone or with atropine, raised in mice the LD50 of dyflos slightly more than did PAM, given alone or with atropine, it failed to do so in the case of sarin (*iso*propyl methylphosphonofluoridate).

In our studies which were carried out in male mice weighing 20 g, atropine was injected i.p. 30 min before, and pro-PAM and PAM i.p. 5 min before Paraoxon (diethyl-4-nitrophenylphosphate), given s.c. Table 1 shows the number of mice dying in 24 hours/number of mice injected.

When mice were injected with atropine sulphate (5 μmol/kg i.p.) 30 min later with Paraoxon (2 μmol/kg s.c.) and after a further 30 min with pro-PAM or PAM (200 μmol/kg i.p.) the AChE activity in the brain, expressed as a percentage of control, 90 and 300 min after the injection of Paraoxon was: 31.0 ± 2.9 (mean ± s.e. mean) and 39.5 ± 3.7 in mice injected with Paraoxon only, 37.0 ± 5.9 and 39.0 ± 4.4 in mice injected with Paraoxon and PAM, and 43.0 ± 3.2 and 64.0 ± 7.5 in mice injected with Paraoxon and pro-PAM.

These findings show that under the experimental conditions chosen, reactivation of phosphorylated AChE in the brain fails to raise markedly the antidotal action obtained by peripheral reactivation alone. This casts doubt on the view that accumulation of acetylcholine in the brain is a major cause of death produced by lipid soluble anticholinesterases. This view is supported by our observation that the enhancement of the antidotal action of oximes by atropine, which often is ascribed in part to a central anti-acetylcholine action, could be duplicated by the poorly lipid soluble atropine methylnitrate.

References

BODOR, N., SHEK, E. & HIGUCHI, T. (1976). Improved delivery through biological membranes. 1. Synthesis and properties of 1-methyl-1,6-dihydropyridine-2-carbaldoxime, a pro-drug of N-methylpyridinium-2-carbaldoxime chloride. *J. Med. Chem.* **19**, 102–107.

Table 1 Antidotal action of pro-PAM and PAM

Dose of paraoxon	*Control*	*Atropine* (5 μmol)	*PAM* (20 μmol)	*pro-PAM* (20 μmol)	*Atropine* (5 μmol) + *PAM* (20 μmol)	*Atropine* (5 μmol) + *pro-PAM* (20 μmol)	*PAM* (200 μmol)	*pro-PAM* (200 μmol)
2 μmol	0/9	0/6	0/6	0/6	—	—	—	—
4 μmol	6/9	0/6	3/6	3/6	1/6	0/6	0/6	0/6
8 μmol	9/9	6/6	6/6	6/6	5/6	4/6	0/6	3/6
20 μmol	—	—	—	—	6/6	6/6	5/6	6/6

All doses represent μmol/kg.

CLEMENT, J.G. (1978). Efficacy of pro-PAM (N-methyl-1,6-dihydropyridine-2-carbaldoxime hydrochloride) as a treatment for organophosphate poisoning. Suffield technical paper No. 487.

HOBBIGER, F. & VOJVODIĆ, V. (1966). The reactivating and antidotal actions of N,N'-trimethylenebis (pyridinium-4-aldoxime) (TMB-4) and N,N'-oxydimethylenebis (pyridinium-4-aldoxime) (Toxogonin) with particular reference to their effect on phosphorylated acetylcholinesterase in the brain. *Biochem. Pharmacol.*, **15**, 1677–1690.

RUTLAND, J.P. (1958). The effect of some oximes in Sarin poisoning. *Brit. J. Pharmac.*, **13**, 399–403.

SHEK, E., HIGUCHI, T. & BODOR, N. (1976). Improved delivery through biological membranes. 3. Delivery of N-methylpyridinium-2-carbaldoxime chloride through the blood-brain barrier in its dihydropyridine pro-drug form. *J. Med. Chem.*, **19**, 113–117.

Prevention of the acquisition of tolerance to eserine in mice

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Buckley & Heading (1978) demonstrated that development of tolerance to neostigmine by rodents could be prevented by the protein synthesis inhibitor cycloheximide. This present report concerns development of tolerance to eserine by mice, and its prevention by cycloheximide and the tubulin binding agent colchicine. Colchicine is not a protein synthesis inhibitor, but can, like cycloheximide, interfere with learning and memory mechanisms (Clingbine & Heading, 1979).

Sensitivity to eserine was assessed by recording a temperature response to a challenge dose (0.5 mg/kg i.p.) of eserine, using a thermistor probe inserted into the rectum to a depth of 0.5 cm. Tolerance was produced by daily injections of eserine (0.5 mg/kg i.p.) 5 days per week for up to three weeks, the last injection being 24 h before challenge. Groups of 5 male Tyler original mice 20–25 g were used, each group being housed separately. Ambient temperature was 18–20°C.

Typically, body temperature of naive animals fell by a maximum of 8.3 ± 0.4°C after challenge and returned to normal after 2 hours. Pretreatment with eserine for 1, 2 and 3 weeks reduced such falls to 6.35 ± 0.8, 3.2 ± 0.5 and 2.8 ± 0.5°C respectively with progressively shorter times taken for recovery.

Using mice receiving eserine for three weeks, along with controls, cycloheximide was injected on day 1 of each week at 1 mg/kg i.p.; the dose shown to prevent neostigmine tolerance in rats (Buckley *et al.*, 1978). Colchicine injections followed the same pattern at 40 μg/kg i.p., a dose shown by Rose & Sinha (1976) to interfere with the synthesis of a particulate brain neuronal fraction whose production is suppressed in visual cortex of rats reared in the dark. Mice receiving eserine alone (group A) showed a maximum fall after challenge of 2.6 ± 0.3, cycloheximide with eserine (group B) a fall of 6.8 ± 1.5 and cycloheximide alone (group C) a fall of 6.8 ± 1.5°C. Mice receiving colchicine with eserine (group D) showed a maximum fall after challenge of 4.6 ± 0.8 and those receiving colchicine alone (group E) a fall of 5.2 ± 0.7°C. Groups B, C, D and E all differed significantly from A indicating that both cycloheximide and colchicine had impaired the development of tolerance. Mean temperatures before challenge were between 35.6 ± 1.3 and 37.1 ± 1.2°C, but no group in the study significantly differed from any other. (All comparisons judged at the 5% level, Student's t test).

Subjective comparisons between groups A, B & D during pretreatment revealed that group A mice ceased to show signs of salivation and muscle fasciculations after one week, while those of groups B & D continued to show such symptoms.

These preliminary results offer no explanation for the mechanism of action of cycloheximide or colchicine, however they do extend the degree of reported overlap between the actions of cycloheximide and colchicine to an effect on tolerance.

References

BUCKLEY, G.A. & HEADING, C.E. (1978). Tolerance to neostigmine and its prevention by cycloheximide treatment. *J. Pharm. Pharmac.*, **30**, 332–334.

CLINGBINE, G. & HEADING, C.E. (1979). Effect of Neurotubule Disrupting Agents on Goldfish Behaviour. *Physiol. Behav.*, **23** (In press).

ROSE, S.P.R. & SINHA, A.K. (1976). Rapidly labelling and exported neuronal protein; [^{3}H]-fucose as a precursor, and effects of cycloheximide and colchicine. *J. Neurochem.*, **27**, 963–966.

The effects of enflurane and halothane upon liver blood flow and oxygen consumption in the greyhound

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Halothane has been in widespread use as a general anaesthetic since its introduction in 1956. In the past few years there has been increasing suspicion that it produces dose related hepatic damage possibly due to a metabolite of the drug (Inman & Mushin, 1978). Enflurane, another volatile hydrocarbon, has recently been introduced in the U.K. and as no cases of 'Enflurane Hepatitis' have been reliably documented (Black, 1979), it has been suggested that this agent could substitute for halothane in 'at risk' patients.

A model has been described in the greyhound in which pentobarbitone anaesthetised greyhounds (25–35 kg) were paralysed with pancuronium bromide and mechanically ventilated to maintain normal blood gas and acid base status (Hughes, Mathie, Campbell & Fitch, 1979).

Hepatic arterial and portal venous blood flows were measured using Statham SP2202 electromagnetic flowmeters. Portal and hepatic venous pressures were measured via indwelling cannulae. These cannulae also allowed portal venous and hepatic venous blood to be sampled for measurement of oxygen content and this with the blood flow values allowed hepatic oxygen consumption to be calculated. Mean arterial blood pressure was recorded continuously and cardiac output was determined by the thermodilution technique. Vascular resistances were calculated using the haemodynamic data obtained.

Two groups of six greyhounds were studied. In the first group four increasing concentrations of halothane were administered for 30 min periods. In the second group four increasing concentrations of enflurane were administered for 30 min periods.

In both groups mean arterial blood pressure decreased linearly with increasing dosage. At equipotent anaesthetic concentrations there was a significantly larger decrease with enflurane than with halothane. Cardiac output decreased linearly by a similar amount in both groups. Hepatic arterial, portal venous and total liver blood flows decreased linearly in a similar manner in both groups. There were insignificant decreases in hepatic oxygen consumption with halothane and no change with enflurane. There were small insignificant decreases in peripheral resistance with halothane while there were significant decreases with enflurane, decreasing to 50% of control with 3% enflurane. Hepatic arterial resistance was unchanged with halothane but decreased significantly with enflurane.

In a wide range of situations when portal venous blood flow is decreased, hepatic arterial resistance also decreases, allowing some autoregulation of hepatic arterial blood flow. This phenomenon is seen with enflurane but not halothane. This suggests hypotension with halothane could potentially produce hypoxia in a liver whose blood supply is already compromised. While enflurane does not have this drawback, its greater cardiovascular depressant effect is possibly a much greater hazard.

References

BLACK, G.W. (1979). Enflurane. *Br. J. Anaesth.*, **51**, 627–640.

HUGHES, R.L., MATHIE, R.T., CAMPBELL, D. & FITCH, W. (1979). Effect of hypercarbia on hepatic blood flow and oxygen consumption in the greyhound. *Br. J. Anaesth.*, **51**, 289–296.

INMAN, W.M. & MUSHIN, W.W. (1978). Jaundice after repeated exposure to halothane. *Br. Med. J.*, **2**, 1455–1456.

Protective role of intestinal and pulmonary enzymes against environmental phenols

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Oral ingestion and pulmonary inhalation are the major routes by which environmental phenols enter the body. We have evaluated *in vivo* the ability of the conjugating enzymes situated in the intestinal mucosa and lung to detoxify these xenobiotics and hence reduce their systemic availability.

Studies were carried out in anaesthetised rats (Sprague-Dawley, male, mean weight 260 g) using (U-^{14}C) phenol (10 μCi/kg) at three different doses (0.4, 1.5 and 4.5 mg/kg). Blood concentration-time profiles for phenol following administration by intra-arterial (via left carotid artery, i.a.), intravenous (via left jugular vein, i.v.) and oral (injection into duodenal lumen, p.o.) routes have been determined. A specific extraction procedure to separate parent compound from its metabolites was applied to each blood sample. The area under the phenol blood concentration-time curves between zero and infinity (AUC) are shown in Figure 1.

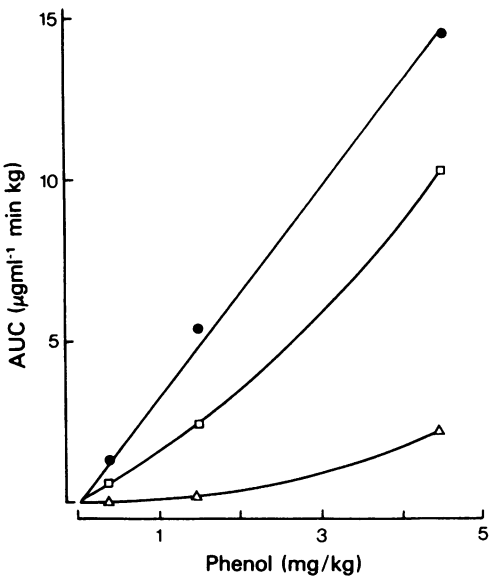

Figure 1 Relationship between area under the phenol blood concentration-time curve between zero and infinity (AUC) and the dose administered for the intra-arterial (●), intravenous (□) and oral (△) routes. Each data point represents the mean of 3–7 animals.

Analysis of variance demonstrated a statistically significant difference ($P < 0.01$) between the phenol AUC for the three routes at each dose. Recovery of total radioactivity demonstrated that the body intake of [^{14}-C] was complete in each case. The marked reduction in AUC after p.o. relative to i.v. may be explained by metabolism during first pass through the intestinal mucosa and liver. Only 0.5–14% of the dose reaches the systemic circulation by this route. Preliminary studies where phenol is administered directly into the hepatic portal vein, and hence by-passes the intestinal mucosa, show that the liver contributes minimally to this effect. Comparison of AUC following i.a. and i.v. allows assessment of pulmonary first pass. After administration into the jugular vein (i.v.) the phenol dose must traverse the lung prior to reaching the arterial system for distribution throughout the body. Pulmonary first pass results in only 40–76% of the dose reaching the systemic circulation.

The concave nature of the i.v. and p.o. AUC-dose plots indicates that the enzyme systems involved in these first pass effects are readily saturable. Weitering, Krijgsheld & Mulder (1979) have recently demonstrated dose dependency in the urinary composition of phenol metabolites in rat following systemic administration of phenol. We are currently investigating the relationship between sites of metabolism and quantitative and qualitative changes in phenol metabolites.

Reference

WEITERING, J.G., KRIJGSHELD, K.R. & MULDER, G.J. (1979). The availability of inorganic sulphate as a rate limiting factor in the sulphate conjugation of xenobiotics in the rat. *Biochem. Pharmac.*, **28**, 757–762.

Rat hepatic microsomal cytochrome(s) P-450 induced by clofibrate

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Phenobarbitone and 3-methylcholanthrene are representative inducers of the mammalian hepatic microsomal cytochrome variants P-450 and P-448 respectively (Testa & Jenner, 1976). This induction is accompanied by increases in the metabolism of specific model substrates. These cytochromes have been shown by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS–PAGE) to consist of multiple forms, the composition-profile of which is influenced by exposure to inducing agents (Welton & Aust, 1974).

Chronic administration of clofibrate and other hypolipidaemic agents (clofenopate and fenofibrate) to rats causes liver enlargement with increases in hepatic cytochrome P-450 and NADPH cytochrome *c* reductase (Orton & Higgins, 1979). However, the metabolism of model substrates was either unaffected or inhibited. We were interested to describe the cytochrome profile following clofibrate administration.

Liver microsomes prepared from control and treated male Alderley Park albino rats (150–180 g) were solubilised and examined by SDS–PAGE as described by Dent, Elcombe, Netter & Gibson (1978).

SDS–PAGE indicated differences in the protein bands in the 50–60,000 molecular weight region (Figure 1). In control microsomes 5 bands were observed in this region. Of the haemoprotein (cytochrome) bands, Band 2 (mol. wt. 52,000) was the densest band, whilst bands 3 (55,000) and 4 (57,000) are barely visible.

Phenobarbitone and the nonplanar molecule 2,4,2',4'-tetrachlorobiphenyl caused a marked increase in Band 2, a slight increase in Band 3 and no change in Band 4.

3-Methylcholanthrene and the planar molecule 3,4,3',4'-tetrachlorobiphenyl caused increases in Bands 3 and 4. Aroclor 1254, a mixture of polychlorinated biphenyls, gave increases in Bands 2, 3 and 4.

Clofibrate increased Bands 2 and 3, a profile differing from that produced by phenobarbitone or 3-methylcholanthrene. A similar profile has been observed after administration of Firemaster BP6, a mixture of polybrominated biphenyls, but unlike clofibrate this compound did increase the hepatic microsomal metabolism of certain model substrates (Dannan, Moore, Besaw & Aust, 1978).

The increase in total cytochrome P-450 during clofibrate administration is due to the formation of specific haemoproteins which may differ catalytically from those induced by other agents.

References

DANNAN, G.A., MOORE, R.W., BESAW, L.C. & AUST, S.D. (1978). 2,4,5,3',4',5-hexabromobiphenyl is both a 3-methylcholanthrene- and a phenobarbital-type inducer of microsomal drug metabolising enzymes. *Biochem. Biophys. Res. Commun.*, **85**, 450–458.

DENT, J.G., ELCOMBE, C.R., NETTER, K.J. & GIBSON, J.E. (1978). Rat hepatic microsomal cytochrome(s) P-450 induced by polybrominated biphenyl. *Drug Metab. Dispos.*, **6**, 96–101.

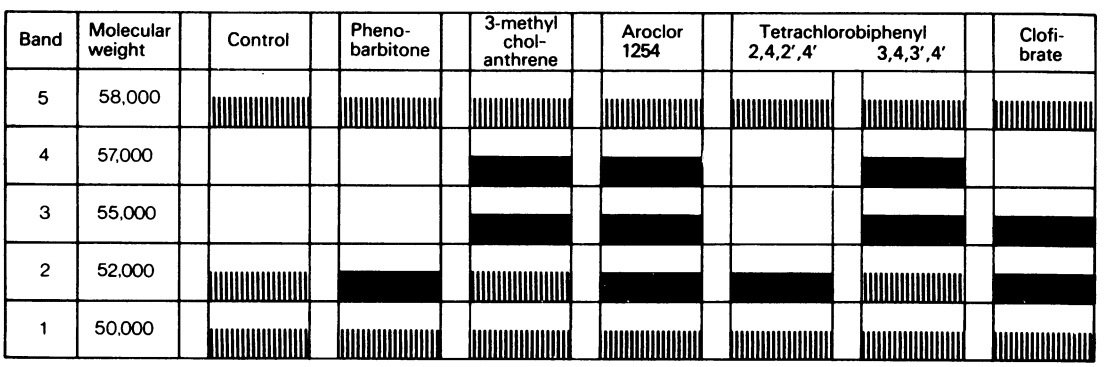

Figure 1 Diagrammatic representation of the major protein staining bands between 50,000 and 58,000 mol. wt. following treatment with inducing agents. Control: Saline or Arachis Oil, Phenobarbitone (75 mg kg^{-1} day^{-1}, i.p. for 3 days), 3-Methylcholanthrene (20 mg kg^{-1} day^{-1}, i.p. for 3 days), Aroclor 1254 (500 mg/kg, i.p. killing on day 6), 2,4,2',4' and 3,4,3',4' Tetrachlorobiphenyl (41 mg kg^{-1} day^{-1} i.p. for 3 days), Clofibrate (0.4% w/w in diet for 14 days)

ORTON, T.C. & HIGGINS, J.E. (1979). The effect of hypolipidaemic agents on liver drug metabolising enzyme. *Toxicol. Appl. Pharmacol.*, **48**, A126.

TESTA, B. & JENNER, P. (1976). Drug Metabolism: Chemical and Biochemical Aspects, Marcel Dekker Inc, New York.

WELTON, A.F. & AUST, S.D. (1974). Multiplicity of cytochrome P-450 haemoproteins in rat liver microsomes. *Biochem. Biophys. Res. Commun.*, **56**, 898–906.

The effect of hypolipidaemic agents on hepatic peroxisomal and microsomal drug metabolising enzymes in relation to thyroxine modulation

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Clofibrate and other hypolipidaemic agents cause liver enlargement and proliferation of hepatic smooth endoplasmic reticulum (SER) and peroxisomes in the rat (Hess, Staubli & Reiss, 1965). The free acid of clofibrate is highly protein bound and increases the liver: serum ratio of tracer thyroxine (Ruegamer, Ryan, Richert & Westerfeld, 1969). Mitochondrial α-glycerophosphate dehydrogenase acts as a specific marker for the metabolic activity of thyroxine in the liver (Lee, Takemori & Lardy, 1959). The present study examines the relationship between thyroxine disposition by hypolipidaemic drugs and the activity of peroxisomal enzymes and the endoplasmic reticulum drug metabolising enzymes (DME).

Male hooded rats (initial weight 150–170 g) were fed the hypolipidaemic drugs in powdered diet for 14 days. Livers were homogenised in sucrose/phosphate buffer and mitochondrial and microsomal fractions prepared by differential centrifugation.

Peroxisomal palmitoyl-CoA oxidation (PCO) and catalase (CAT) were measured in total homogenates and α-glycerophosphate dehydrogenase (GPD) in the mitochondrial fraction; cytochrome P-450 content, aminopyrine demethylation (APDM) and ethoxyresorufin-*O*-deethylation (ERDE) were assayed in the microsomal fraction.

The effects of the drugs on hepatic enzymes are shown in Table 1. All treatments caused increases in liver/body weight ratios and in GPD reflecting increased thyroxine activity in the liver. The activity of catalase and of the peroxisomal fatty-acyl CoA oxidation system were also increased.

Cytochrome P-450 content was markedly increased indicating proliferation of SER. The activity of the DME (insignificant increase in APDM and decrease in ERDE) did not parallel the increases in cytochrome P-450. In the case of Atromid, the low activity of APDM is not due to the presence of the drug as APDM and P-450 levels at 48 and 72 h after dosing are still altered although drug was not detectable in the blood. ERDE had, however, returned to control levels at this time.

Table 1 Effect of hypolipidaemic agents on activity of hepatic peroxisomal and microsomal enzymes

*Drug** / *Assay*	% *Control*† *Clofibrate* (0.4%)	*Lipantyl* (0.05%)	*Tibric acid* (0.05%)
Aminopyrine N-demethylation	120	91	125
Ethoxyresorufin O-deethylation	50	26	73
Cytochrome P450	148	224	218
Palmitoyl-CoA oxidation	1568	2247	392
Catalase	151	134	136
α-Glycerophosphate dehydrogenase	375	377	782
Liver:Bodyweight	163	151	175

* Male hooded rats (3 per group) were administered the drugs in powdered diet at the doses indicated for 14 days. Control animals received drug free powdered diet.
† Enzyme assays were performed in duplicate and the activity for treated animals is expressed as a % of control activity.

Hypolipidaemic agents of varying structures have characteristic effects on hepatic peroxisomal enzymes and microsomal DME. The expression of which may be mediated at least in part by thyroxine disposition as evidenced by increases in hepatic α-GPD.

References

HESS, R., STAUBLI, W. & RIESS, W. (1965). Nature of the hepatomegalic effect produced by ethyl chlorophenoxyisobutyrate in the rat. *Nature*, **208**, 856–858.

LEE, Y.P., TAKEMORI, A.E. & LARDY, H. (1959). Enhanced oxidation of α-glycerophosphate by mitochondria of thyroid fed rats. *J. Biol. Chem.*, **234**, 3051–3054.

RUEGAMER, W.R., RYAN, N.T., RICHERT, D.A. & WESTERFELD, W.W. (1969). The effects of *p*-chlorophenoxyisobutyrate on the turnover rate and distribution of thyroid hormone in the rat. *Biochem. Pharmacol.*, **18**, 613–624.

The effect of lead exposure on brain catecholamines and their synthetic enzymes in the rat

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Studies of the correlation of neurochemical and behavioural effects of lead in animals are both inadequate and contradictory (Shih & Hanin, 1978). In the present preliminary study, the neurochemical correlates of lead exposure in the rat have been investigated by the measurement of steady state levels of catecholamines and the activity of catecholamine synthesizing enzymes.

Three groups of male Wistar rats (100 g) were injected daily for 14 days with saline or lead (5 or 20 μmol/kg). Rats were killed by decapitation, the brains excised, and the cortex, pons medulla, hippocampus and anterior and posterior hypothalamus were sectioned. The concentrations of lead were measured in the various regions of the brain by flameless atomic absorption spectrophotometry. Noradrenaline (NA) and adrenaline (AD) concentrations were determined by the method of Prada & Zurcher (1976) whilst the activities of tyrosine hydroxylase (TH) and phenylethanolamine N methyl transferase (PNMT) were determined by the method of Petty & Reid (1979). Results were analysed by analysis of variance and the Scheffé test. Lead pretreatment of the rats was associated with significant increases in lead levels in all the brain regions studied. In the anterior and posterior hypothalamus increases in NA levels in the lead treated animals were associated with decreases in TH activity (Table 1). PNMT activity in both regions was unaffected by lead treatment whilst AD levels in anterior hypothalamus were significantly elevated in the 20 μmol/kg lead group. In other regions there were no significant changes in the levels of catecholamines and the activity of catecholamine synthesizing enzymes.

The decrease in TH activity, the rate limiting enzyme of catecholamine synthesis, and the accompanying rise in NA concentration in both anterior and posterior hypothalamus, demonstrate that lead

Table 1 The effect of lead treatment on catecholamines and catecholamine synthesizing enzymes in anterior and posterior hypothalamus

	Posterior hypothalamus			*Anterior hypothalamus*		
	Control	*Lead (5 μmol/kg)*	*Lead (20 μmol/kg)*	*Control*	*Lead (5 μmol/kg)*	*Lead (20 μmol/kg)*
Pb (nmol/mg protein)	3.6 ± 0.4	5.1 ± 2.7	15.3 ± 8.8**	3.8 ± 1.9	6.2 ± 3.5	13.4 ± 7.0**
TH activity (nmoles[^{14}C]Dopa/mg protein/h)	186 ± 61	146 ± 77	142 ± 44	164 ± 32	116 ± 41	105 ± 30*
NA (nmol/mg protein)	5.8 ± 2.9	9.2 ± 3.2	10.4 ± 2.1*	7.0 ± 2.0	14.0 ± 2.9**	17.0 ± 3.6**
PNMT activity (pmoles[^{3}H]N methyl phenyl ethanolamine/mg protein/h	20.1 ± 7.2	26.6 ± 16.1	24.2 ± 11.9	20.5 ± 7.6	18.9 ± 6.3	21.1 ± 11.1
AD (nmol/mg protein)	0.33 ± 0.23	0.30 ± 0.20	0.56 ± 0.45	0.45 ± 0.37	0.54 ± 0.41	2.2 ± 1.5*

Each figure represents mean ± S.D. *$P < 0.05$. **$P < 0.01$.

exposure leads to marked changes in central noradrenergic neuronal activity. It is proposed to examine these changes further in more discrete areas of the brain.

References

PETTY, M.A. & REID, J.L. (1979). Catecholamine synthesizing enzymes in brain stem and hypothalamus during the development of renovascular hypertension. *Brain Res.*, **163**, 277–288.

PRADA, M.D. & ZURCHER, G. (1976). Simultaneous radioenzymatic determination of plasma and tissue adrenaline and noradrenaline within the femtomole range. *Life Sci.*, **19**, 1161–1174.

SHIH, T.M. & HANIN, I. (1978). Chronic lead exposure in immature animals: Neurochemical correlates. *Life Sci.*, **23**, 877–888.

Stereoselective excretion of 3-methoxy-4-hydroxyphenylethylene glycol sulphate in the dog

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Studies on the peripheral and central adrenergic mechanisms continue to receive considerable attention. A non-invasive technique for assessing central noradrenaline turnover in man would be of particular value, both clinically and in research. Noradrenaline is almost completely metabolised before leaving the CNS, the major end product being 3-methoxy-4-hydroxyphenylethylene glycol (MHPG) (Sjostrom, Ekstedt & Anggard, 1975). However, since MHPG is also formed peripherally, measurement of plasma or urinary MHPG levels will not provide an index of central amine turnover. Since the ratio of MHPG sulphate to glucuronide, the major urinary conjugates, produced in the periphery is very different from that in the CNS (Karoum, Moyer-Schwing, Potkin & Wyatt, 1977) determination of the ratio of peripheral conjugates, after infusion of MHPG, and of total conjugates should enable the central contribution to be calculated. As a preliminary to infusion of MHPG into man studies were conducted in greyhound dogs.

MHPG labelled with six deuterium atoms (d_6-MHPG) was synthesised in four stages from deuterated acetovanillone. The product, which was purified by preparative thin layer chromatography was a colourless, viscous oil. MHPG and its conjugates in urine were assayed as previously described and in plasma by a modification of this method with d_2-MHPG and d_3-MHPG sulphate as internal standards (Murray, Baillie & Davies, 1977). Approximately 1 mg of d_6-MHPG in sterile saline was infused into a foreleg vein of conscious greyhound dogs at a constant rate over 5–6 hours. Blood samples were taken hourly up to 7 h and then at 24 h and 48 hours. Urine was collected by catheter over short intervals up to 7 h and then to 24 h and 48 hours.

Plasma levels of endogenous MHPG, MHPG sulphate and MHPG glucuronide did not change for the duration of the infusion. For d_6-MHPG the free compound and its glucuronide achieved steady state plasma levels between 2–3 hours. Plasma concentrations of d_6-MHPG sulphate increased continuously during the infusion. The renal clearances of endogenous MHPG glucuronide and sulphate were high at 60–80 ml min^{-1} and 70–100 ml min^{-1} respectively. Free MHPG clearance was only 10–15 ml min^{-1}. Clearances of free d_6-MHPG and its glucuronide were similar to those for the corresponding endogenous compounds. However, clearance of d_6-MHPG sulphate was low at 1 h and decreased during the infusion reaching 17–19 ml min^{-1} at 4.5 hours. Since racemic deuterium labelled MHPG was used for infusion the low clearance of d_6-MHPG sulphate could be due to either a stereochemical or deuterium isotope effect. Infusion with unlabelled, racemic MHPG gave essentially the same results as with the labelled compound. This ruled out any isotope effect. Determination of the (+)- and (−)-isomers of d_6-MHPG sulphate separately in urine (Frank, Nicholson & Bayer, 1978) revealed that the clearance of the natural, (−)-isomer was far higher than that of the (+)-isomer. The very low clearance of the (+)-isomer, only 1–2% of creatinine clearance, could be due to extensive plasma protein binding. Plasma protein binding of MHPG sulphate, determined by equilibrium dialysis, was 55%.

It is concluded that the natural (−)-isomer of MHPG sslphate is excreted stereoselectively by the kidneys. The use of racemic MHPG or noradrenaline in kinetic studies will thus lead to erroneous conclusions.

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References

FRANK, H., NICHOLSON, G.J. & BAYER, E. (1978). Gas chromatographic-mass spectrometric analysis of optically active metabolites and drugs on a novel chiral stationary phase. *J. Chromatog.*, **146**, 197–206.

KAROUM, F., MOYER-SCHWING, J., POTKIN, S.G. & WYATT, R.J. (1977). Presence of free, sulfate and glucuronide conjugated 3-methoxy-4-hydroxy-phenylglycol (MHPG) in human brain, cerebrospinal fluid and plasma. *Brain Res.*, **125**, 333–339.

MURRAY, S., BAILLIE, T.A, & DAVIES, D.S. (1977). A non-enzymic procedure for the quantitative analysis of (3-methoxy-4-sulphoxyphenyl) ethylene glycol (MHPG sulphate) in human urine using stable isotope dilution and gas chromatography-mass spectrometry. *J. Chromatog.*, **143**, 541–551.

SJOSTROM, R., EDSTEDT, J. & ANGGARD, E. (1975). Concentration gradients of monoamine metabolites in human cerebrospinal fluid. *J. Neurol. Neurosurg. Psychiat.*, **39**, 281–290.

Developmental changes in the inducibility of drug metabolising enzymes by 3-methylcholanthrene in hepatic and extrahepatic tissues of the rabbit

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In a previous study (Atlas, Boobis, Felton, Thorgeirsson & Nebert, 1977) tissue and age specific responses to polycyclic aromatic hydrocarbon inducers were demonstrated in the rabbit. Complex developmental changes in the pattern of hydrocarbon induction of at least two different forms of cytochrome P-450 were the effects of hydrocarbon pretreatment on several non-cytochrome P-450 dependent enzymes of drug metabolism in rabbits of different ages.

New Zealand white rabbits aged −1 d (1 day before expected birth date), 3 d, 7 d, 30 d and 90 d were used. Treated animals received 80 mg Kg^{-1} 3-methylcholanthrene (3MC) in arachis oil i.p. 72 h prior to sacrifice. −1 d old animals were induced by treating the does. Microsomal and cytosolic fractions of liver, lungs and kidneys were prepared as previously described (Atlas, *et al*, 1977). Subcellular fractions were stored at −80°C until assayed. Protein, cytochrome P-450 content, and activity of AHH (Atlas *et al*, 1977), epoxide hydratase (Jerina, Dansette, Lu & Levin, 1976), glutathione S-transferase (Pacifici, Boobis, Brodie & Davies, 1979) and naphthol and morphine glucuronyl transferases (Bock, Brunner, Hoensch, Huber & Josting, 1978) were all assayed by previously reported methods.

Cytochrome P-450 content was increased after 3MC treatment in the liver and kidney at all ages. Significant increases occurred in the lung only at 7 d or younger. AHH activity was highly inducible in the liver and lung of 7 d and younger animals and in kidney at all ages. Styrene oxide hydratase was slightly inducible in liver and lung of younger animals but not in the kidney. At −1 d liver activity was increased from 1.83 ± 0.20 nmol mg^{-1} min^{-1} to 2.66 ± 0.18 nmol mg^{-1} min^{-1} (mean ± s.e. mean) by 3MC treatment and in the lung from 0.24 ± 0.01 nmol mg^{-1} min^{-1} to 0.32 ± 0.01 nmol mg^{-1} min^{-1}. The two glucuronyl transferases were studied only in the liver. Both activities were inducible in younger animals but whereas naphthol conjugation at −1 d increased from 3.38 ± 0.90 nmol mg^{-1} min^{-1} to 14.44 ± 1.66 nmol mg^{-1} min^{-1} morphine conjugation increased from 0.82 ± 0.18 nmol mg^{-1} min^{-1} to only 1.92 ± 0.30 nmol mg^{-1} min^{-1}. The soluble enzyme glutathione S-styrene oxide transferase was slightly inducible in the liver of younger animals and in the kidney of adult animals. At −1 d liver activity increased from 0.93 ± 0.01 nmol mg^{-1} min^{-1} to 1.64 ± 0.12 nmol mg^{-1} min^{-1}.

3MC treatment of rabbits thus causes changes in a number of drug metabolising enzymes in addition to cytochrome P-450. The liver is the organ most sensitive to induction. Younger rabbits are more responsive to induction than older animals thus suggesting that this species differs from most others which are relatively resistant to induction during the foetal and neonatal period.

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References

ATLAS, S.A., BOOBIS, A.R., FELTON, J.S., THORGEIRSSON, S.S. & NEBERT, D.W. (1977) Ontogenetic expression of polycyclic aromatic compound-inducible monooxygenase activities and forms of cytochrome P-450 in rabbits. *J. biol. Chem.*, **252**, 4712–721.

BOCK, K.W., BRUNNER, G., HOENSCH, H.. HUBER, E. & JOSTING, D. (1978) Determination of microsomal UDP-glucuronyltransferase in needle-biopsy specimens of human liver. *Eur. J. clin. Pharmac.*, **14**, 367–373.

JERINA, D.M., DANSETTE, P.M., LU, A.Y.H. & LEVIN, W. (1976) Hepatic microsomal epoxide hydrase: a sensitive radiometric assay for hydration of arene oxides of carcinogenic aromatic hydrocarbons. *Molec. Pharmacol.*, **13**, 342–351.

PACIFICI, G.M., BOOBIS, A.R., BRODIE, M.J. & DAVIES, D.S. (1979) A rapid sensitive assay for glutathione s-epoxide transferase activity: species differences in the activity of hepatic enzymes. *Biochem. Soc. Trans.*, in press.