POSTER COMMUNICATIONS

Modification of the uterotrophic responses to oestradiol by mepyramine and inhibitors of prostaglandin synthesis

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Prostaglandins and histamine have been suggested as mediators of the oestrogen induced increase in uterine blood flow in the ovariectomized rat (Phaily & Senior, 1978; Spaziani, 1975). Evidence for the involvement of these agents in the early water imbibition and uterotrophic effects of oestradiol is incomplete (Brandon, 1978). To investigate further such involvement experiments were performed in female rats (weight range, 300–350 g), ovariectomized at least 3 weeks prior to the experiment. Uterine blood flow was measured using radioactive microspheres (15 μm diameter) in the sodium pentobarbitone (60 mg/kg i.p.) anaesthetized rat. The uterus was dissected free from mesentery, blotted and weighed wet. The tissue was then weighed after drying to constant weight in an oven. Initial experiments established a maximum increase in uterine blood flow from 52 ± 13 ml min^{-1} 100 g^{-1} uterine wet weight to 810 ± 150 ml min^{-1} 100 g^{-1} 3 h after injection of 0.5 μg/kg oestradiol-17β into the tail vein of restrained rats. Significant increases in uterine wet and dry weights were observed 3 h after oestradiol injection with maximum increase in wet weight at 9 hours.

Neither of the prostaglandin synthesis inhibitors, indomethacin 1 mg/kg orally or AH 7170 (2-m-(p-chlorobenzoyl)phenyl-propionic acid) (Glaxo Research Ltd) 1 mg/kg orally, nor the histamine H_1 receptor antagonist, mepyramine maleate 5 mg/kg i.p., had any significant effect on the oestrogen induced increase in blood flow 3 h after the oestrogen treatment. Inhibition of prostaglandin synthesis or histamine H_1 receptor antagonism significantly inhibited the increase in uterine wet weight at 3 h and dry weight increase at 9 h following oestrogen treatment. Actinomycin D 0.5 mg/kg i.p. significantly inhibited oestrogen induced increase in both wet and dry weights of the uterus but did not affect the blood flow response. Resnik *et al.* (1975) also found that actinomycin D did not inhibit oestrogen induced blood flow in the uterus of the sheep. The results suggest that the increase in blood flow to the uterus in the rat 3 h after oestradiol injection is not dependent on prostaglandin synthesis, histamine H_1 receptor stimulation or ribo-nucleic acid synthesis. These mechanisms, however, are probably involved in the early oestrogen water imbibition response of the uterus and in subsequent uterine growth.

References

BRANDON, J.M. (1978). Failure of indomethacin and D-2-bromolysergic acid diethylamide to modify the acute oedematous reaction to oestradiol in the uterus of the immature rat. *J. Endocr.*, **78**, 291–292.

PHAILY, S. & SENIOR, J. (1978). Modification of oestrogen induced hyperaemia by drugs in the overiectomized rat. *J. Reprod. Fert.*, **53**, 91–97.

RESNIK, R., BATTAGLIA, F.C., MAKOWSKI, E.L. & MESCHIA, G. (1975). The effect of actinomycin D on estrogen induced uterine blood flow. *Am. J. Obstet. Gynec.*, **122**, 273–277.

SPAZIANI, E. (1975). Accessory reproductive organs in mammals: control of cell and tissue transport by sex hormones. *Pharmacol. Rev.*, **27**, 207–286.

Effect of ICI 55897 (CLOZIC*) and indomethacin on type II collagen-induced arthritis in the rat

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Collagen-induced arthritis in rats was originally reported by Trentham, Townes & Kang (1977), where they described the development of arthritis in the hind feet of inbred (Wistar-Lewis) or outbred (Wistar, Sprague–Dawley) female rats following i.d. injections of a mixture of incomplete Freunds adjuvant and type II collagen (articular). Arthritis could be produced by type II collagen from either rat, chick or human sources but not by type I or type III collagen.

We have produced collagen-induced arthritis in male Alderley Park albino rats by giving them i.d. injections of bovine type II collagen emulsified with incomplete Freunds adjuvant. Using 0.5 mg type II collagen per rat an acute inflammatory arthritis developed in one or both hind feet 10 to 14 days after injection. The acute arthritis subsided in approximately 70% of rats and had disappeared after 4 weeks; persistent chronic arthritis developed in approximately 30% of rats. Progression of the arthritis was characterized by measuring four parameters, (i) hind foot swelling by mercury displacement, (ii) serum glycoprotein (α1GP) by radial immunodiffusion, this protein is useful for following the time course of arthritis in the rat and also provides a sensitive measure of drug efficacy (Billingham & Gordon, 1976), (iii) serum anti-type II collagen antibody levels were measured by solid-phase radioimmunoassay (Clague, Brown, Weiss & Holt, 1979) and finally (iv) sections of arthritic rat feet were examined histologically to ascertain which tissues were involved in acute and chronic lesions. The first three parameters were used to compare the activity of indomethacin and a new anti-arthritic agent ICI 55897.

For these experiments, type II collagen arthritis was induced in groups of at least 15 rats, as described above; separate groups received either indomethacin (2 mg kg daily) or ICI 55897 (20 mg kg daily), orally, and further groups were kept as untreated arthritic controls. With prophylactic treatment, commencing two days before induction of the arthritis, both agents were found to inhibit development of the arthritis, seen as an inhibition of foot swelling and an inhibition of the rise of serum α1GP levels, in comparison with control. Similarly when rats with established arthritis were treated from day 14 onwards, both agents returned the foot swelling and elevated serum α1GP levels to near normal values. Neither agent had any effect on the titre of serum anti-type II collagen antibody levels.

Indomethacin and ICI 55897 are effective inhibitors of the inflammatory swelling and the rise in α1GP levels associated with collagen-induced arthritis in the rat. These drugs have a similar inhibitory effect in adjuvant-induced arthritis in the rat (Billingham, 1980), and this together with other experiments which demonstrate that the time course and histopathology of both collagen arthritis and adjuvant arthritis have considerable similarity (unpublished findings), suggest there are common factors in the aetiology of these models of arthritis.

* CLOZIC is a trademark the property of Imperial Chemical Industries Ltd.

References

BILLINGHAM, M.E.J. (1980) Anti-arthritic properties of ICI 55897 (CLOZIC). Future Trends in inflammation I.V., (In Press).

BILLINGHAM, M.E.J. & GORDON A.H. (1976). The role of the acute phase reaction in inflammation. *Agents and Actions*, **6**, 195–200.

CLAGUE, R.B., BROWN, R.A., WEISS, J.B. & HOLT, P.J.L. (1979). Solid-phase radioimmunoassay for the detection of antibodies to collagen. *J. Immunol. Meth.*, **27**, 31–41.

TRENTHAM, D.E., TOWNES, A.S. & KANG, A.H. (1977). Auto-immunity to type II collagen: an experimental model of arthritis. *J. Exp. Med.*, **146**, 857–867.

The effect of the nonsteroidal anti-inflammatory agent, diflunisal, on energy metabolism in mitochondria isolated from rat liver

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In the course of an investigation on the properties of diflunisal (2',4'-difluoro-4-hydroxy-[1,1'-biphenyl]-3-carboxylic acid) we have tested the effect of the compound on oxidative phosphorylation in isolated liver mitochondria.

Tightly-coupled rat liver mitochondria exhibited the phenomenon of respiratory control, i.e. when the mitochondria were respiring in the presence of succinate, phosphate and oxygen (state 4), then the addition of ADP elicited a stimulation of respiration (state 3) which returned to control levels when the ADP had been converted to ATP. In the presence of diflunisal (3.3–33 μM), the ADP response remained, but the stimulated rate failed to return to control levels. Since this progressive loss of respiratory control can be due to an uncoupling action, we examined the effect of diflunisal on state 4 respiration and ATP hydrolysis. Both reactions were stimulated by diflunisal at low concentrations (3.3–66 μM): at higher concentrations (66–132 μM) an inhibition of the stimulated reaction occurred, particularly in the case of state 4 respiration (Table 1). IC_{50} values (concentration required to give 50% of the maximal rate) were similar to those obtained with the uncoupling agent, 2,4-dinitrophenol, and were (diflunisal values given first; $n = 5$) 14.1 ± 3.5 μM and 10.5 ± 3.1 μM for state 4 respiration, and 12.4 ± 1.2 μM and 7.5 ± 0.1 μM for ATP hydrolysis. Similar results were obtained when succinate was replaced with glutamate plus malate.

Confirmation of an uncoupling action was obtained when it was shown that the inhibition of state 3 respiration by the energy-transfer inhibitor, oligomycin (0.33–1.67 μg/ml) was released by diflunisal (3.3–66 μM), that the diflunisal-stimulated ATPase was blocked by oligomycin.

The present study shows that diflunisal is capable of inhibiting the formation of ATP and promoting its hydrolysis by uncoupling oxidative phosphorylation. This property is also found in a number of other anti-inflammatory agents (Tokumitsu, Lee & Ui, 1977).

P. McD. is an S.R.C. student.

References

BEECHEY, R.B. (1966). The uncoupling of respiratory chain phosphorylation by 4,5,6,7-tetrachloro-2-trifluoromethylbenzimidazole. *Biochem. J.*, **98**, 284–289.

TOKUMITSU, K., LEE, S. & UI, M. (1977). *In vitro* effects of nonsteroidal anti-inflammatory drugs on oxidative phosphorylation in rat liver mitochondria. *Biochem. Pharmac.*, **26**, 2101–2106.

Table 1 Effect of diflunisal on state 4 respiration and ATP hydrolysis in tightly coupled rat liver mitochondria

Diflunisal (μM)	*State 4 respiration* (*ngatom oxygen/min/mg protein*)	*ATP hydrolysis* (*nmol phosphate released/ min/mg protein*)
0	22.4 ± 0.8	25.2 ± 1.5
3.3	24.6 ± 0.5	31.1 ± 1.7
6.6	27.9 ± 0.3	39.5 ± 1.3
16.5	39.0 ± 1.1	67.3 ± 3.2
33	44.5 ± 0.9	80.1 ± 6.5
66	49.2 ± 1.5	78.5 ± 7.9
132	36.5 ± 3.4	76.1 ± 4.9

State 4 respiration was measured using an oxygen electrode. The reaction chamber contained 0.25 M sucrose, 10 mM tris-HCl buffer, pH 7.4, 3.3 mM sodium succinate and 3.3 mM phosphate buffer, pH 7.4 at 30 . The reaction was initiated by the addition of rat liver mitochondria (10 mg protein) to the chamber, to give a final volume of 3 ml; diflunisal was added 2 min after the mitochondria. ATPase activity was estimated by the method of Beechey (1966). Values are presented as mean ± s.e. mean ($n = 5$).

The effect of pituitary hormones on hepatic drug metabolism

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Sex differences exist in the hepatic microsomal metabolism of lidocaine (a local anaesthetic and anti-arrhythmic drug) (von Bahr, Hedlund, Karlén, Bäckström & Grasdalen, 1977) and imipramine (a tricyclic anti-depressant) (Bickel & Gigon, 1971) in the rat. The male animal exhibits a higher lidocaine N-deethylation and imipramine N-demethylation and N-oxidation whereas the female shows a higher 3-hydroxylation of lidocaine and 2-hydroxylation of imipramine.

Recent evidence has suggested that these sex differences are under dual control—from the pituitary (via the gonads) in the male and apparently directly from the pituitary in the female (Skett, Mode & Gustafsson, 1979). This is a similar situation to that found for steroid metabolism in the rat liver where a novel pituitary factor (the 'feminizing factor') has been postulated to exist. The 'feminizing factor' is only secreted by the pituitary in the female animal and maintains the female-type metabolism in the liver (Skett & Gustafsson, 1979).

In order to ascertain the role of 'feminizing factor' in the control of drug metabolism, a series of experiments were designed based on the administration of extracts of pituitary glands or standard pituitary hormones to male rats using the Alzet® osmotic mini-pump. The mini-pump provides a continuous infusion of hormone over seven days without disturbing the animal. Pituitary extract, growth hormone, prolactin, follicle-stimulating hormone, thyroid-stimulating hormone and D-Ala-D-Leu-enkephalin (BW180C) were tested.

Pituitary extract gave a feminization of lidocaine and imipramine metabolism. Growth hormone, prolactin and BW180C mimicked the effect of pituitary extract on lidocaine N-deethylation but not on lidocaine 3-hydroxylation. The effect of pituitary extract on imipramine N-oxide was mimicked by FSH but this hormone had the opposite effect to the pituitary extract on imipramine N-demethylation.

Although most of the effects of the pituitary extract could be mimicked by standard pituitary hormones, no one hormone accounted for the feminization seen with the pituitary extract. This suggests that the feminizing effect was due to a hormone not tested or a complex mixture of those tested.

The pituitary hormones were the kind gift of the National Institute of Arthritis, Metabolism and Digestive Diseases and BW180C was kindly supplied by Dr. S. Wilkinson, The Wellcome Research Laboratories.

References

VON BAHR, C., HEDLUND, I., KARLÉN, B., BÄCKSTRÖM, D. & GRASDALEN, H. (1977). Evidence for two catalytically different binding sites of liver microsomal cytochrome P-450: Importance for species and sex differences in oxidation patterns of lidocaine. *Acta Pharmacol. et Toxicol.*, **41**, 39.

BICKEL, M.H. & GIGON, P.L. (1971). Metabolic interconversions and binding of imipramine, imipramine-N-oxide and desmethylimipramine in rat liver slices. *Xenobiotica*, **1**, 631.

SKETT, P., MODE, A. & GUSTAFSSON, J-Å. (1979). Hormonal control of hepatic drug metabolism. XIth International Congress of Biochemistry, Toronto, Abstract No. 11-6-S149.

SKETT, P. & GUSTAFSSON, J.Å. (1979). Imprinting of enzyme systems of xenobiotic and steroid metabolism. *Rev. Biochem. Toxicol.*, **1**, 27.

Immunosuppressive effects of vinyl chloride monomer on delayed hypersensitivity in the guinea-pig

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Vinyl chloride or its metabolites have been shown to exert toxic effects in tissues of a number of rodents (Mastromatteo, Fisher, Christie & Danziger, 1960) and alkylating properties have been demonstrated by Green & Hathway, (1979). Whole body autoradiography of [^{14}C] vinyl chloride in rats showed that, apart from concentration in organs of metabolism and elimination plus some glandular structures, accumulation occurred in the thymus (Green & Hathway, 1975). In view of the importance of the thymus in relation to functions of the immune system and the likely cytotoxicity of vinyl chlroide, an investigation of possible immunosuppression was prompted.

Both humoral and cell-mediated immune reactivity were investigated simultaneously in the guinea-pig, using a variation of the approach of Street & Sharma (1975). Antibody production was stimulated by single injections of crystalline egg albumen (CEA) (1 mg intraperitoneal) in aqueous solution emusified in equal parts of Freunds' complete adjuvant (FCA) (Hicks & Skeldon, 1970). Antibody was measured by passive haemagglutination and complement fixation. Delayed skin hypersensitivity to *M. tuberculosis* (0.5 mg) in the FCA (type H37 Ra, Difco) was assessed by reactions provoked by intradermal injections of tuberculin P.P.D. (50 μg). Numbers of circulating leucocytes were counted at intervals during and after treatment and sensitization. Secondary immune responses were investigated after a further administration of the combined CEA/FCA antigens, 10 weeks after primary sensitization.

Oral administration of vinyl chloride (70 mg/kg, daily) in corn oil, for 28 days prior to primary sensitization with the combined antigen, caused significant suppression of delayed skin hypersensitivity and suppressed antigen-induced rises in leucocyte counts but had no effect on titres of antibody. Both primary and secondary immune mechanisms were affected by the preliminary treatments but other courses of VCM treatment, during and after primary or secondary sensitizations were much less effective.

Inhibition of the cell-mediated tuberculin hypersensitivity was associated with suppression of elevations in numbers of circulating small lymphocytes and neutrophils but the slower changes in medium and large lymphocytes were unaffected. The role of the thymus in relation to cell-mediated immune functions is not limited to the neonatal period. In young adult animals it continues to serve as a major source of lymphoid cells (Ernstrom & Larsson, 1967; Cronkite & Chanana, 1970). It is suggested that the accumulation of vinyl chloride or its possibly cytotoxic metabolites in this tissue may account for the small-cell lymphocytopenia and the associated suppression of cell-mediated immune reactivity.

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References

CRONKITE, E.P. & CHANANA, A.D. (1970). Lymphocytopoiesis. In '*Formation and destruction of blood cells*', ed. Greenwalt, T.J. & Jamieson, G.A., pp. 284–306. Philadelphia: J.B. Lippincott.

ERNSTROM, V. & LARSSON, B. (1967). Export and import of lymphocytes in the thymus during steroid-induced involution and regeneration. *Acta Path. Microbiol. Scand.*, **70**, 371–386.

GREEN, T. & HATHWAY, D. (1975). The biological fate in rats of vinyl chloride in relation to its oncogenicity. *Chem. Biol. Interact.*, **11**, 545–562.

GREEN, T. & HATHWAY, D.E. (1977). The chemistry and biogenesis of the S-containing metabolites of vinyl chloride in rats. *Chem. Biol. Interact.*, **17**, 137–150.

HICKS, R. & SKELDON, N. (1970). The influence of adjuvant on antibody production and anaphylactic hypersensitivity in the guinea-pig. *Int. Arch. Allergy*, **34**, 470–478.

MASTROMATTEO, E., FISHER, A.M., CHRISTIE, H. & DANZIGER, H. (1960). Acute inhalation toxicity of vinyl chloride to laboratory animals. *Amer. indust. Ass. J.*, **21**, 394–396.

STREET, J.C. & SHARMA, R.P. (1975). Alteration of induced cellular and humoral immune response by pesticides and chemicals of environmental concern: quantitative studies of immunosuppression by D.D.T., Arochlor 1254, carbaryl, carbofuran and methylparathion. *Toxical. Appl. Pharmacol.*, **32**, 587–602.

Do the recurrent laryngeal nerves provide an extrinsic input to non-adrenergic inhibitory neurones in guinea-pig trachealis muscle?

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The *in vivo* experiments of Chesrown, Venugopalan, Gold & Drazen (1980) suggested that the intramural non-adrenergic inhibitory neurones of guinea-pig trachea (Coburn & Tomita, 1973; Coleman & Levy, 1974; Clark, 1978) receive extrinsic neural input carried in the recurrent laryngeal nerves. To test this suggestion we have developed an isolated preparation of cervical trachea in which we have compared responses to transmural stimulation with those evoked by stimulation of an extrinsic segment of the recurrent laryngeal nerve.

Tracheae were excised from guinea-pigs (450–750 g, either sex) together with the recurrent laryngeal nerves and segments of the vagi. Connections of the recurrent laryngeal nerve with the lower trachea were severed but those made with cervical tissue were kept intact. Extrinsically innervated segments of cervical trachea were set up for recording of mechanical ac-

tivity (Coburn & Tomita, 1973) under isotonic conditions (load 200 mg).

Stimulation (5–20 V, 16–32 Hz, 0.5 ms pulse width, 10 s train duration) of the extrinsic recurrent laryngeal nerve was evoked by a pair of electrodes placed around the nerve and more than 1 cm from the tracheal segment. Field stimulation (100 V, 16–32 Hz, 0.5 ms pulse width, 5 or 10 s train duration) was applied using a pair of electrodes 1 cm apart and located parallel to the trachealis and on either side of the tissue. The two types of stimulation were performed alternately.

In normal Krebs' solution both field and extrinsic nerve stimulation evoked contractions which were suppressed by atropine (10^{-6} M). The contractile response to extrinsic nerve stimulation was abolished by hexamethonium (5×10^{-4} M) or by cutting the recurrent laryngeal nerve. The contractile response to field stimulation was unaffected by either of these procedures.

Atropine-treated tissues relaxed in response both to extrinsic nerve stimulation and to field stimulation. Relaxant responses to extrinsic nerve stimulation were generally small and were unaffected by hexamethonium. However, they were abolished by cutting the recurrent laryngeal nerve, by guanethidine (5×10^{-5} M) and by propranolol (2.5×10^{-6} M). Relaxant responses to field stimulation were larger, were unaffected by hexamethonium or extrinsic nerve section but were reduced by guanethidine or propranolol.

The recurrent laryngeal nerves hence appeared to carry both cholinergic and noradrenergic fibres innervating the cervical trachealis. However we obtained no evidence to suggest that the recurrent laryngeal nerves carried extrinsic neural input to the non-adrenergic inhibitory fibres of the cervical trachealis. Extrinsic input to these fibres either does not occur or is provided by an alternative route.

L.C. is a CASE student.

References

CHESROWN, S.E., VENUGOPALAN, C.S., GOLD, W.M. & DRAZEN, J.M. (1980). *In vivo* demonstration of non-adrenergic inhibitory innervation of the guinea-pig trachea. *J. Clin. Invest.*, **65**, 314–320.

CLARK, L.A. (1978). Studies of the innervation of guinea-pig trachealis. M.Sc. Thesis University of Manchester.

COBURN, R.F. & TOMITA, T. (1973). Evidence for non-adrenergic inhibitory nerves in the guinea-pig trachealis muscle. *Am. J. Physiol.*, **224**, 1072–1080.

COLEMAN, R.A. & LEVY, G.P. (1974). A non-adrenergic inhibitory nervous pathway in guinea-pig trachea. *Br. J. Pharmac.*, **52**, 167–174.

Some actions of furazolidone in poultry

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Furazolidone has recently been shown to inhibit monoamine oxidase (MAO) activity in the chicken (Ali & Bartlet, 1980), and, in the present experiments, in turkey poults and ducklings. When administered by crop tube furazolidone was about equipotent in inhibiting MAO activity in these three species (Figure 1).

Inclusion of furazolidone in the feed at a concentration of 0.04% w/w for 10 days produced anorexia in chickens, turkey poults and ducklings, but no mortality. The treatment produced a similar degree of MAO inhibition in the hearts, brains and duodenal mucosae from the three species, enzymatic activity being inhibited by 29 to 59% ($P < 0.05$ to < 0.001). Only in the duckling was a significant inhibition of MAO activity found in the liver ($P < 0.01$). Furzolidone 0.04% w/w for 10 days is the therapeutic treatment for galliformes (Veterinary Data Sheet, 1980), the recommended dose for ducks being 0.01% w/w of the drug for 7 days.

Monoamines extracted from brain with perchloric acid were purified on a cation exchange resin (Amberlite CG-50, ammonium form). 5-Hydroxytryptamine (5-HT) was estimated by its fluorescence in 3 M HCl and catecholamines by the trihydroxyindole method. The concentration of 5-HT in brains of ducklings fed furazolidone (0.04% w/w for 10 days) was 0.57 ± 0.06 μg/g tissue (mean ± s.e. mean, 5 birds), and in paired controls 0.39 ± 0.04 μg/g. This increase in brain 5-HT ($P < 0.05$) was selective, as concentrations of adrenaline and noradrenaline in the brain were unchanged after feeding furazolidone to the ducklings ($P > 0.1$).

The pressor actions of graded doses of tyramine and noradrenaline were measured in chickens anaesthetized with phenobarbitone sodium (180 mg/kg, i.v.). Responses to tyramine in preparations made from chickens fed furazolidone (0.04% w/w for 10

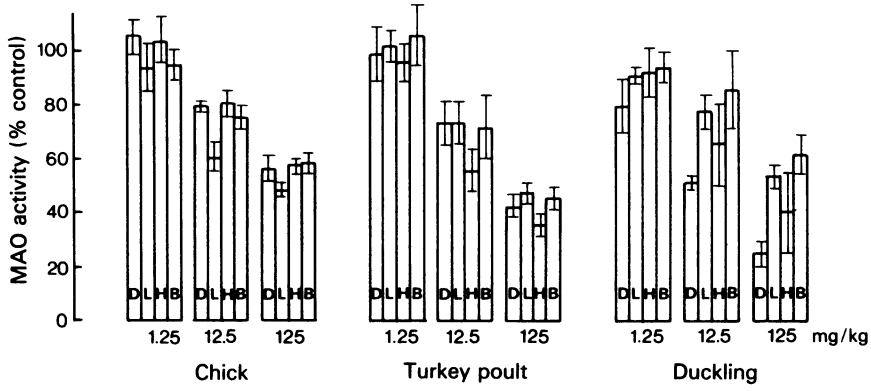

Figure 1 The inhibition of monoamine oxidase (MAO) activity in chicks, turkey poults and ducklings 24 h after furazolidone administered by crop tube. Ordinate: MAO activity estimated by the method of Krajl (1965), expressed as a percentage of the value in control birds. Abscissa: doses of furazolidone (mg/kg) administered to birds aged 2 to 12 days. The columns represent mean values for duodenal mucosa (D), liver (L), heart (H) or brain (B) from 5 chicks, 5 turkey poults and 3 ducklings; the vertical bars at the head of the columns represent the s.e. Furazolidone inhibited MAO activity in the tissues of the three species to about the same extent, except that in the heart it was greater in turkey poults than in chicks ($P < 0.05$) and in duodenal mucosa more marked in ducklings than in chicks or turkey poults ($P < 0.05$).

days) were about 70% greater than those in control preparations. The pressor action of noradrenaline was unaffected by the treatment.

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References

ALI, B.H. & BARTLET, A.L. (1980). Inhibition of monoamine oxidase by furazolidone in the chicken and the influence of the alimentary flora thereon. *Br. J. Pharmac.*, In the press.

KRAJL, M. (1965) A rapid microfluorimetric determination of monoamine oxidase. *Biochem. Pharmac.*, **14**, 1684–1685.

An electrophysiological study of the effects of ionophore A23187 in an insect salivary gland

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Calcium dependent activation of potassium channels has been reported in a number of preparations (Meech, 1978) and it has been suggested that in the salivary gland cells of the cockroach, *Nauphoeta cinerea*, Olivier, receptor activation by dopamine results in an increase in the intracellular calcium ion concentration which then increases the potassium permeability of the acinar cell membrane, producing a hyperpolarisation (Ginsborg, House & Mitchell, 1980).

The ionophore A23187, which transports calcium ions (Reed & Lardy, 1972), has been employed in the present study and had been found to produce a hyperpolarisation due to an increase in membrane potassium permeability. In the presence of calcium ions in the bathing fluid, a large, prolonged hyperpolarisation was evoked but in the absence of extracellular calcium ions, the ionophore was able to produce a hyperpolarisation, which, in general, was

smaller than that evoked in calcium containing medium and was transient.

The results support the idea that an increase in the potassium permeability arises from an increase in the intracellular calcium ion concentration. It is suggested that the ionophore evoked a transient response in the absence of extracellular calcium due to mobilisation of calcium from a store in the acinus.

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References

GINSBORG, B.L., HOUSE, C.R. & MITCHELL, MARY R. (1980). A calcium-readmission response recorded from *Nauphoeta* salivary gland acinar cells. *J. Physiol.* (In Press).

MEECH, R.W. (1978). Calcium-dependent potassium activation in nervous tissues. *Ann. Rev. Biophys. Bioeng.*, **7**, 1–18.

REED, P.W. & LARDY, H.A. (1972). A23187: A divalent cation ionophore. *J. Biol. Chem.* **247**, 6970–6977.

Changes in the rat intestine exposed to polyethylene glycol 2000 and polyoxyethylene (40) stearate

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Polyethylene glycols (PEG) have been used in the formulation of drugs with poor dissolution rates in aqueous media such as griseofulvin (Chiou & Riegelman, 1970).

Kaur, Grant & Eaves (1979) have shown *in vitro* that the dissolution rates of a variety of drugs including griseofulvin were improved when formulated with polyoxyethylene (40) stearate (Myrj 52). No adverse morphological effect has been attributed to Myrj 52 although at high concentrations it does have a laxative effect (WHO, 1974).

A number of studies have indicated that intestinal damage is associated with exposure to PEGs (Kameda, Abei, Nastallahn & Iber, 1968; Clarke & Kobayashi, 1975). In this study a comparison was made of the effects of PEG 2000 and Myrj 52 on (a) the morphological integrity of the intestinal barrier and (b) fluid movement across the barrier.

Adult male Wistar rats, fed on a standard Pilsburg 41B diet were anaesthetized with pentobarbitone i.p. and the abdomen opened with a mid-line incision. Test solutions at concentrations of 1%, 10%, 20%, 30% of PEG 2000 in 0.9% saline and Myrj 52 in 0.9% saline were introduced into closed gut segments prepared from a portion of ileum free of intestinal contents and left for either 30 or 60 min; the segments were then divided, fixed and processed for microscopy. Similar segments were filled with 0.9% saline, or left unfilled and processed as control tissue. Fluid movement was assessed using 1 ml solutions of the excipients or saline containing phenol red (16.5 mg/l) introduced into similar closed gut segments. Samples were taken throughout the experiment and the phenol red concentration measured.

At concentrations of the excipients up to 20% no histological changes were detected in the intestinal mucosa. Sham and saline segments had a normal histology, and all segments were without evidence of ischaemic damage. Above concentrations of 20% there was evidence of extensive cell loss from the apicies of the intestinal villi. The loss appeared greater in segments exposed to PEG and was associated with a crenated villus outline, shortening of villus profile, and the appearance of a group of goblet cells in the epithelium over the highest part of the villus.

In the saline segments there was a loss of intestinal fluid, while in the Myrj 52 segments there was little change in the intestinal volume. The PEG solutions caused a dramatic increase in lumenal fluid and segments containing 30% PEF 2000 were visibly distended. The fluid gain was directly related to concentration and time.

The experiments reported here clearly show that in this form of preparation Myrj 52 and PEG 2000 at high concentrations induce cell loss over a 60 min period and that in the case of PEG the loss results in a characteristic accumulation of goblet cells at the villus apex similar to that observed in the more protracted experiments of Clarke & Kobayashi (1975).

References

CHIOU, W.L. & RIEGELMAN, S. (1970). Absorption characteristics of solid dispersed and micronised griseofulvin in man. *J. Pharm. Sci.*, **60**, 1376–1380.

CLARKE, R.M. & KOBAYASHI, S. (1975). The cytological effects of infusion of lumenal polyethylene glycol on the rat small intestinal mucosa. *Arch. Histol. Jap.*, **38**, 135–150.

KAMEDA, H., ABEI, T., NASRALLAH, S. & IBER, F.L. (1968). Functional and histological injury to the intestinal mucosa produced by hypertoxicity. *Am. J. Physiol.*, **214**, 1090–1095.

KAUR, R., GRANT, D.J.W. & EAVES, T. (1979). Solid dispersions of drugs in polyoxyethylene (40) stearate: dissolution rates and physicochemical interactions. *J. Pharm. Pharmacol.*, **31**, Suppl. 48.

WORLD HEALTH ORGANIZATION (1974). Toxicological evaluation of some food additives including anticaking agents, antimicrobials, antioxidents, emulsifiers and thickening agents. **5**, 264–269.

Catecholamines have a dual action on stomach strips: the relaxation component is selectively inhibited by domperidone

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Whilst there have been extensive studies on dopamine agonist and antagonist action in psychiatry and neurology, the effects of these drugs to modify gastrointestinal, particularly stomach, motility has been recorded almost in passing, although some agents such as metoclopramide and domperidone have been developed for clinical use to facilitate gastric emptying (De Schepper, Wollaert & Reyntjens, 1978; Reyntjens, Niemegeers, Van Neuten, Laduron, Heykants, Schellekens, Marsboom, Jageneau, Brokaert & Janssen, 1978). The suggestion that there may exist dopamine receptors in the stomach to mediate the observed changes is a logical extrapolation, but has received limited experimental support (Valenzuela, 1976; Van Neuten, Ennis, Helsen, Laduron & Janssen, 1978). Hence, in a series of investigations we have been concerned with establishing the nature of changes caused by dopamine agonists and antagonists in the upper gastro-intestinal tract, and report here on changes caused by catecholamines on three stomach segments, cardia, fundus and body, using domperidone as a putative dopamine antagonist.

Male Dunkin-Hartley guinea-pigs weighing 350–450 g were killed by cervical trans-section and smooth muscle strips (15 mm × 5 mm) from the cardia, fundus and body regions of the stomach isolated. The strips were dissected in a plane suitable to investigate tension changes in the circular muscle layer. The mucosal layer was removed and the tissue bathed in 15 ml oxygenated 95% O_2; 5% CO_2) Krebs and Hensleit solution at 37 C containing 100 mg/l ascorbic acid. Tension changes were detected by Grass tension transducers and the response area integrated (Illingworth & Naylor, 1980) in addition to display on a multichannel Grass recorder. One gram tension was applied to the tissues which were allowed to equilibrate for 30–45 min before the addition of drugs.

Both noradrenaline (10^{-7}–10^{-5} M) and dopamine (2×10^{-5}–2×10^{-4} M) caused marked relaxation of all three gastric strips. At subthreshold doses noradrenaline (10^{-8}–10^{-7} M), and to a greater extent dopamine (4×10^{-7}–10^{-5} M), contracted the cardia, fundus and body. In the presence of propranolol (10^{-7} M) plus phentolamine (10^{-6} M) both relaxation and contraction responses to noradrenaline and dopamine were abolished. Domperidone (10^{-7} and 10^{-6} M) partially antagonised the relaxation induced by noradrenaline and dopamine but did not significantly alter the contraction responses. However, domperidone (10^{-5} M) almost completely abolished the relaxation caused by both agonists and significantly potentiated the contraction phase which was thus apparent over a much wider dose range. The contraction induced by noradrenaline and dopamine in the presence of domperidone (10^{-5} M) was antagonised by phentolamine (10^{-6} M).

In a further study the above concentrations of the α- plus β-adrenoceptor antagonists were shown to completely abolish the relaxation caused by isoprenaline and phenylephrine. However, domperidone (10^{-5} M) failed to antagonize the response to isoprenaline although that to phenylephrine was abolished.

The present studies establish two important points, firstly that noradrenaline and, in particular, dopamine can cause contraction of stomach circular smooth muscle and relaxation at higher doses. Secondly, that whilst both effects can be antagonized by phentolamine, domperidone selectively inhibits the relaxation phase to facilitate muscular contraction of the stomach.

References

DE SCHEPPER, A., WOLLAERT, F. & REYNTJENS, A. (1978). Effects of oral domperidone on gastric emptying and motility. *Arzneim-Forsch.*, **28**, 1196–1199.

ILLINGWORTH, D.R. & NAYLOR, I.L. (1980). An inexpensive, electronic digital integrator suitable for use with isolated tissues. *J. Pharmac. Meth.*, in press.

REYNTJENS, A.J., NIEMEGEERS, C.J.E., VAN NEUTEN, J.M., LADURON, P., HEYKANTS, J., SCHELLEKENS, K.H.L., MARSBOOM, R., JAGENEAU, A., BROEKAERT, A. & JANSSEN, P.A.J. (1978). Domperidone, a novel and safe gastrokinetic anti-nauseant for the treatment of dyspepsia and vomiting. *Arzneim-Forsch.*, **28**, 1194–1195.

VALENZUELA, J.E. (1976). Dopamine as a possible neurotransmitter in gastric relaxation. *Gastroenterology*, **71**, 1019–1022.

VAN NEUTEN, J.M., ENNIS, C., HELSEN, L., LADURON, P.M. & JANSSEN, P.A.J. (1978). Inhibition of dopamine receptors in the stomach: an explanation of the gastrokinetic properties of domperidone. *Life Sci.*, **23**, 453–458.

Evidence for a novel mechanism of action of salmon calcitonin on indomethacin-induced gastric erosions

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We have recently described a potent action of salmon calcitonin to inhibit the development of indomethacin-induced gastric erosions in the rat (Bates, Buckley & Strettle, 1980). In the present series of experiments we have investigated the role of gastric acid in the development of indomethacin-induced erosions and compared the antierosive activity of calcitonin with that of drugs which reduce gastric acidity.

Gastric erosions were induced in starved (24 h) Sprague–Dawley (C.D. strain) rats of either sex, over a 5 h period, by the administration of indomethacin (40 mg/kg i.p.). The gastric pH was measured as previously described (Bates, Buckley & Strettle, 1979) and the erosions were stained by a modification of the method of Robert & Nezamis (1964). Salmon calcitonin, cimetidine and atropine were administered s.c. at the same time as indomethacin. Dijex® (a proprietary antacid preparation, containing aluminium hydroxide gel (0.98 g/ml) and magnesium hydroxide (17 mg/ml) Boots Co.) was administered 0, 100 and 200 min after indomethacin.

Neither cimetidine (3.3–200 mg/kg) nor atropine (10 mg/kg) significantly affected the development of indomethacin-induced erosions (Figure 1). The antacid preparation (10 ml/kg intragastrally 0, 100 and 200 min after indomethacin) also did not significantly affect the development of erosions (Figure 1) but elevated the gastric pH from 2.6 in control animals (distilled water 0, 100 and 200 min after indomethacin) to 5.3 in antacid treated animals.

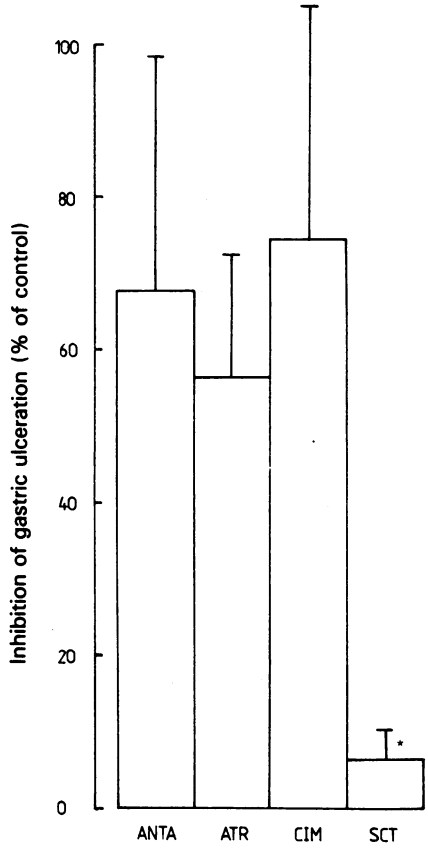

Figure 1 A comparison of the maximally effective doses of antacid (ANTA), atropine (ATR) (10 mg/kg), cimetidine (CIM) (50 mg/kg) and salmon calcitonin (SCT) (10 MRC u/kg). Values are expressed as a percentage of the appropriate control values. Means + s.e. mean. $n = 6$. Significant differences between control and drug treated animals are shown at the $P < 0.05$ level (*) (Mann-Whitney U test).

Calcitonin (0.1–100 MRC u/kg) gave a significant inhibition of erosion development (Figure 1).

Despite the use of doses of cimetidine (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975) and atropine which have been shown to markedly inhibit gastric acid secretion and doses of antacid which significantly elevated gastric pH, the development of indomethacin-induced erosions could not be inhibited by these compounds.

Similarly pepsins are unlikely to make a major contribution to the development of erosions as they have been shown to be inactivated at pHs above 5 (Konturek, 1976).

In contrast to drugs which reduce gastric acidity, calcitonin gave up to a 95% inhibition of the development of erosions. Thus it would appear that in addition to any effect due to the previously described actions of calcitonin to inhibit acid (Bobalik, Kleszynski, Aldred & Bastian, 1974) and pepsin (Orimo, Oyama & Ito, 1973) secretions, calcitonin must be acting by a previously undescribed mechanism to inhibit indomethacin-induced erosion formation.

The salmon calcitonin used in this study was kindly supplied by Drs J.W. Bastian & J.P. Aldred, Armour Pharmaceutical Corp., Illinois, USA.

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References

BATES, R.F.L., BUCKLEY, G.A. & STRETTLE, R.J. (1980). The action of salmon calcitonin on indomethacin-induced gastric ulceration in the rat. *Br. J. Pharmac.*, **67**, 483–484P.

BOBALIK, G.R., KLESZYNSKI, R.R., ALDRED J.P. & BASTIAN, J.W. (1974). Differential effects of salmon, porcine and human calcitonin on gastric secretion and gastric emptying in rats. *Proc. Soc. Exp. Biol. Med.*, **147**, 284–288.

BRIMBLECOMBE, R.W., DUNCAN, W.A.M., DURANT, G.J., EMMETT, J.C., GANELLIN, C.R. & PARSONS, M.E. (1975). Cimetidine—A non-thiourea H_2-receptor antagonist. *J. Int. Med. Res.*, **3**, 86–92.

KONTUREK, S.J. (1976) Gastric secretion. In: M.T.P. International Review of Science **4** Gastrointestinal Physiology ed. Jacobson, E.D. & Shanbour, L.L. Butterworths: London pp. 227–264.

ORIMO, H., OYAMA, T. & ITO, H. (1973). Calcitonin inhibition of gastric secretion in rat. *Endocrinol. Jpn.*, **20**, 439–446.

ROBERT, A. & NEZAMIS, J.E. (1964). Histopathology of steroid-induced ulcers. *Arch. Pathol. Lab. Med.*, **77**, 407–423.

Gastric acid secretion in the rabbit isolated mucosa

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The aims of the experiments described here were to assess the suitability of this preparation for concentration response studies and to investigate histamine receptors in the rabbit gastric mucosa.

Male New Zealand White Rabbits (1.0–1.5 kg) which had been allowed free access to food and water were killed by a blow to the neck. The stomach was removed and the antrum excised. The fundic portion was cut along the lesser curvature and washed in cold saline. The tissue was pinned to a cork board and the musosa separated by the injection of oxygenated saline between the mucosa and the muscle layers. The 'blistered' mucosa was stripped away using a scalpel, and pieces tied over the ends of short perspex tubes (i.d. 1.13 cm), mucosal surface inwards. The preparations were set up at 34°C in separate baths containing 30 ml of a buffered saline solution (110 mM NaCl; 5 mM KCl; 0.5 mM $CaCl_2$; 1.2 mM $MgCl_2.6H_2O$; 26 mM $NaHCO_3$; 16.7 mM glucose) gassed with 95% O_2, 5% CO_2. Drugs were added to the bath. 5.0 ml of unbuffered solution (136 mM NaCl; 5 mM KCl; 0.5 mM $CaCl_2$; 1.2 mM $MgCl_2.6H_2O$; 16.7 mM glucose) gassed with 100% O_2 was added to the mucosal side. The mucosal solution was removed every 15 min and titrated to pH 7 with 0.01 M NaOH.

Depending upon the rates of spontaneous secretion the preparations could be broadly divided into two categories: Those which were low (1–2 $\mu MH^+ cm^{2-1} h^{-1}$) spontaneous secretors and which responded reliably in the concentration response studies and those which maintained a high (4–8 $\mu MH^+ cm^{2-1} h^{-1}$) rate of spontaneous secretion and in which a stimulated secretion could not be obtained.

In responsive tissues histamine (6×10^{-6}–3×10^{-4} M), methacholine (2.6×10^{-6}–2.6×10^{-5} M) and the specific H_2-receptor agonist impromidine (5×10^{-7}–2×10^{-5} M) produced concentration-related increases in acid secretion. ED_{50} values were, for histamine 5×10^{-5} M, methacholine 8×10^{-6} M, impromidine 3.4×10^{-6} M. Impromidine was ap-

proximately 15 times more potent than histamine on this preparation but its maximal response was only 67% of that to histamine ($n = 6$). The maximum secretory rates obtained were in the range 6–8 $\mu MH^+ cm^{2-1} h^{-1}$.

Metiamide (1×10^{-4} M) caused a parallel shift to the right in the histamine concentration effect curve but had no significant effect on the response to methacholine. Atropine (1×10^{-5} M) abolished the methacholine responses. Mepyramine (2.5×10^{-6} M) had no effect on the histamine concentration effect curve ($n = 6$) and in addition the specific H_1-agonist 2-(2-aminoethyl) thiazole dihydrochloride (2-TEA) did not stimulate acid secretion ($n = 5$).

In 'high' basal secretors Metiamide (1×10^{-4} M) reduced the rate of spontaneous acid secretion. However neither 2-TEA nor mepyramine had any effect on this spontaneous secretion.

These results indicate that the rabbit isolated fundic mucosa is a viable preparation which can be used for concentration response studies *in vitro* and suggest that if histamine H_1-receptors are indeed present on the mucosa they do not appear, at least in the case of the rabbit, to be involved in the regulation of gastric acid secretion.

Histidine decarboxylase activity in the isolated gastric mucosa of the rat

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Histamine H_2-receptor antagonists *in vivo* produce an increase in gastric mucosal histidine decarboxylase (HDC) activity in the rat, (Maudsley *et al.*, 1974; Malinski & Sewing, 1977) and augment pentagastrin-induced enzyme activation (Hakanson *et al.*, 1977). However, these changes are probably the consequence of gastrin release caused by the inhibition of gastric acid secretion (Hakanson, 1975). For this reason, studies were carried out on an isolated gastric mucosa preparation which enabled the effects of secretagogues and antagonists on HDC activity to be investigated in the absence of any indirect hormonal activity.

Male, Wistar rats (approx. 150 g) were anaesthetized with pentobarbitone (6 mg/100 g body weight). The gastric mucosa was separated from the muscle layers using the 'blistering' technique described by Forte *et al.* (1975). The mucosa was mounted in an organ-bath according to the method of Main & Pearce (1978) and the mucosal surface superfused with unbuffered Krebs–Henseleit solution (1 ml/min). The acid output was measured as described by Bunce & Parsons (1976).

Pentagastrin was added to the serosal solution. On achieving the maximal response to pentagastrin, the mucosa was removed from the organ bath, blotted dry, weighed and homogenized in ice-cold 0.1 M Tris–maleate buffer (pH 7.0) to a final concentration of 100 mg/ml.

The HDC assay was a modification of the method of Hakanson *et al.* (1974) using L-histidine-[^{14}C]OOH as substrate.

The effect of pentagastrin (3×10^{-8} M–3×10^{-6} M) on acid secretion and HDC activity were investigated in absence and presence of metiamide (3×10^{-5} M and 10^{-3} M).

The basal level of acid secretion in unstimulated gastric mucosa was 17.3 ± 1.2 nmol/min (means ± s.e. mean, $n = 24$) and the corresponding HDC activity in the mucosa was 5.2 ± 0.2 pmol CO_2 mg tissue^{-1} h^{-1} ($n = 24$).

A bell-shaped dose–response curve to pentagastrin was obtained in the dose range 3×10^{-8} M–3×10^{-6} M. The maximum secretory response was achieved with 3×10^{-7} M pentagastrin (60.0 ± 17.9 nmol/min, $n = 9$) after 16.4 ± 1.2 min ($n = 9$) of drug contact.

Pentagastrin (3×10^{-8} M–3×10^{-6} M) stimulated a significant increase in HDC activity over basal levels, ($P < 0.001$). This increase did not appear to be dose-related although the HDC activity stimulated by 3×10^{-6} M (12.9 ± 1.6 pmol CO_2/mg tissue/h, $n = 4$), was significantly greater than that obtained in presence of 3×10^{-8} M pentagastrin (7.9 ± 1.1 pmol CO_2/mg tissue/h, $n = 4$) ($P < 0.05$).

Metiamide (3×10^{-5} M, 10^{-3} M) had no significant effect on the basal levels of acid output (12.4 ± 3.0 nmol/min, $n = 11$ and 13.6 ± 2.6 nmol/min, $n = 20$, respectively) or HDC activity (5.2 ± 0.6 pmol CO_2/mg tissue/h, $n = 11$ and 5.4 ± 0.3 pmol CO_2/mg tissue/h, $n = 20$, respectively).

The pentagastrin-induced increase in HDC activity was abolished in the presence of metiamide (3×10^{-5} M and 10^{-3} M) ($P < 0.05$–$P < 0.001$).

The failure of metiamide to increase HDC activity *in vitro* supports the suggestion that its effect *in vivo* is an indirect consequence of inhibition of gastric acid secretion leading to gastrin release. It does appear however, that pentagastrin can stimulate HDC activity, an effect mediated via histamine H_2-receptors.

References

BUNCE, K.T. & PARSONS, M.E. (1976). A quantitative study on metiamide, a histamine H_2-antagonist, on the isolated whole rat stomach. *J. Physiol.*, **258**, 453–465.

FORTE, J.G., FORTE, T.M. & MACHEN, T.E. (1975). Histamine-stimulated hydrogen ion secretion by an *in vitro* piglet gastric mucosa. *J. Physiol.* **244**, 15–31.

HAKANSON, R., HEDENBRO, J., LIEDBERG, F., EL MUNSHID, H.A. & REHFELD, J.F. (1977). Gastrin obligatory intermediate in the postprandial mobilisation of gastric histamine in the rat. *Experentia*, **33**, 1541–1543.

HAKANSON, R., KROESEN, J.H., LIEDBERG, G., OSCARSON, J., REHFELD, J.F. & STADIL, F. (1974). Correlation between serum gastric concentration and rat stomach histidine decarboxylase activity. *J. Physiol.*, **243**, 483–498.

HAKANSON, R., LIEDBERG, G. & REHFELD, J.F. (1975). Activation of histidine decarboxylase by H_2-receptor blockade. Mechanism of action. *Br. J. Pharmac.*, **53**, 127–130.

MAIN, I.H.M. & PEARCE, J.B. (1978). A rat isolated gastric mucosal preparation for studying the pharmacology of gastric secretion and the synthesis or release of endogenous substances. *J. Pharmacol.*, **Methods 1**, 27–38.

MASLINSKI, S. & SEWING, K.F. (1977). Effect of cimetidine on gastric mucosal histamine and histidine decarboxylase in rats. *Digestion*, **15**, 121–128.

MAUDSLEY, D.V., KOBAYASHI, Y., BOVAIRD, L. & ZEIDEL, M. (1974). Effect of H_2-receptor antagonists on histidine decarboxylase activity in rat gastric mucosa. *Biochem. Pharmac.*, **23**, 2963–2968.

Effects of sodium replacement and ouabain on rat gastric mucosal potential difference

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The rat isolated gastric mucosa (Main & Pearce, 1978) has previously been used to investigate the pharmacology of acid secretion. We have now studied the resting transmucosal potential difference (p.d.) and its relationship to sodium transport.

Paired mucosae from rats weighing 80 to 120 g were mounted on vessels with either the mucosal or serosal side uppermost, and placed in organ baths containing buffered solution at 37°C. The upper surface was superfused with identical solution at 12 ml/h. Potential difference was measured via matched calomel electrodes and transmucosal resistance was calculated from the change in p.d. in response to application to a known external current.

Potential difference rose rapidly during the first hour, reaching -24.1 ± 2.1 and -26.1 ± 1.9 mV at 2 and 3 h respectively ($n = 20$). Corresponding values of resistance were 125.0 ± 6.1 and 119.9 ± 5.4 Ω/cm. Ouabain (10^{-4} M) had no effect on p.d. when applied to the mucosal surface for 1 h, but decreased p.d. to $42.8 \pm 4.0\%$ ($n = 5$) of the pretreatment level when applied serosally. In experiments in which the mucosal surface was superfused with unbuffered solution, ouabain inhibited histamine-induced acid secretion when applied serosally but not mucosally.

Replacement of sodium chloride with choline chloride (110 mM) and sodium bicarbonate with Hepes buffer (10 mM), in both solutions, caused an initial rapid decrease in p.d., followed by a slower decline leading to an inversion ($+7.4 \pm 1.6$ mV, $n = 7$, after 30 min) which was abolished by anoxia, raising the temperature to 45°C or restoring sodium. The inversion of p.d. by choline in the absence of sodium may have resulted from stimulation of acid secretion, since, when the mucosal surface was superfused with unbuffered solution, choline (110 mM) increased acid secretion (from 3.4 ± 1.0 and 4.4 ± 1.1 to 3.5 ± 1.1 and 21.5 ± 4.8 μmol cm^{-2} h^{-1} in control and test respectively, after 30 min, $n = 8$) and this effect was blocked by atropine (3×10^{-6} M). In the presence of atropine, choline substitution reduced p.d. to $52 \pm 18\%$ ($n = 3$) of the resting value.

These results provide further evidence for the presence of an electrogenic sodium pump in the rat gastric mucosa (Cummins & Vaughan, 1965) but suggest that it is located on the serosal side of the membrane and may be responsible for only 50% of the resting mucosal p.d.

A.W.B. is a CASE award student.

References

CUMMINS, J.T. & VAUGHAN, B.E. (1965). Ionic relationships of the bioelectrogenic mechanism in the isolated rat stomach. *Biochim. biophys. Acta*, **94**, 280–292.

MAIN, I.H.M. & PEARCE, J.B. (1978). A rat isolated gastric mucosal preparation for studying the pharmacology of gastric secretion and the synthesis or release of endogenous substances. *J. Pharmac. Methods*, **1**, 27–38.

A comparison of the effects of some calcium antagonists on drug-induced rhythmic contractions of the rat vas deferens

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We have previously reported that verapamil antagonises Ca^{2+} contractures in the K^+-depolarized rat vas deferens in lower concentrations than are required to reduce drug-induced rhythmic contractions (Hay & Wadsworth, 1980). We now report results with some other calcium antagonists in this preparation.

Vasa deferentia from Wistar rats (250–450 g body weight) were suspended in Krebs–Henseleit solution (Na^+ = 144, K^+ = 5.8, Ca^{2+} = 2.5, Mg^{2+} = 1.2, HCO_3^- = 25, $H_2PO_4^-$ = 1.2, SO_4^{2-} = 1.2, Cl^- = 129, glucose = 11.1 mM) at 36 to 37°C and contractions were recorded isometrically. Methoxamine HCl (2 μg/ml) or barium chloride (1 mM) produced rhythmic contractions that persisted for several hours.

Nifedipine (0.5–2 μg/ml) reduced the amplitude of rhythmic contractions induced by methoxamine or barium. At 2–5 μg/ml, nifedipine reduced their frequency and then abolished them. Nifedipine resembled verapamil (Hay & Wadsworth, 1980) in that it was considerably less potent in inhibiting rhythmic contractions than KCl-induced contractions in the rat vas deferens.

Diazoxide in low concentrations (1–150 μg/ml, with considerable variation between preparations) reduced the amplitude of methoxamine and barium-induced rhythmic contractions. However, higher concentrations of diazoxide (150–500 μg/ml) augmented the size of barium-induced contractions, and caused the methoxamine-induced contractions to become grouped.

Flunarizine HCl reduced the amplitude of barium-induced rhythmic contractions in concentrations of 5–50 μg/ml, with higher concentrations being required to reduce their frequency. Flunarizine (5–20 μg/ml) also had an inhibitory effect on methoxamine-induced rhythmic contractions, but this effect was difficult to evaluate because the solvent (tartaric acid) also caused inhibition.

In contrast to nifedipine, diazoxide, flunarizine and verapamil, the predominant effect of methoxyverapamil HCl (D600) was on the frequency of rhythmic contractions. Methoxyverapamil (0.4 μg/ml) reduced the frequency of methoxamine-induced rhythmic contractions, while the concentration required to reduce the amplitude was roughly 10 times higher.

Sodium nitroprusside was more active in inhibiting rhythmic contractions than in reducing 160 mM KCl contractions. The amplitude of methoxamine-induced rhythmic contractions was reduced by nitroprusside (5–20 μg/ml) and this effect seemed to reach a plateau at about 50% inhibition. These contractions usually became grouped in the presence of nitroprusside (50–500 μg/ml). Nitroprusside had a biphasic effect on barium-induced rhythmic contractions: at about 20 μg/ml they were inhibited (nitroprusside was slightly less active against barium than against methoxamine) and above 150 μg/ml they were augmented. The effects of nitroprusside on rhythmic contractions resembled those of diazoxide.

The effects of verapamil (Hay and Wadsworth, 1980) and nifedipine could indicate that the calcium channel operated by methoxamine or barium in the rat vas deferens required higher concentrations of the organic calcium antagonists to block than does the calcium channel operated by depolarisation with KCl. However, the effect of methoxyverapamil is more comparable to the inhibition of spontaneously contracting muscle such as the portal vein (Golenhofen & Lammel, 1972) or uterus (Reiner & Marshall, 1975). Nitroprusside had a selective effect on rhythmic contractions, possibly suggesting thay they depend on a mechanism similar to that involved in noradrenaline contractions in the aorta or carbachol contractions in the trachea (Kreye, Baron, Lüth & Schmidt-Gayk, 1975).

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References

GOLENHOFEN, K. & LAMMEL, E. (1972). Selective suppression of some components of spontaneous activity in various types of smooth muscle by iproveratril (verapamil). *Pflügers Arch.*, **311**, 233–243.

HAY, D.W.P. & WADSWORTH, R.M. (1980). Effect of verapamil on rhythmic contractions in isolated rat vasa deferentia. *Br. J. Pharmac.*, **68**, 182P–183P.

KREYE, V.A.W., BARON, G.D., LÜTH, J.B. & SCHMIDT-GAYK, H. (1975). Mode of action of sodium nitroprusside on vascular smooth muscle. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **288**, 381–402.

REINER, O. & MARSHALL, J.M. (1975). Action of D-600 on spontaneous and electrically stimulated activity of the parturient rat uterus. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **290**, 21–28.

The effect of Mg^{2+}-free Krebs solution on agonist responses in the bisected vas deferens of the rat

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In studies with the isolated vas deferens different groups of workers have used bath media of differing composition, particularly with respect to Mg^{2+} concentrations. In some studies the solutions contained Mg^{2+} (Jenkins *et al.*, 1977; Anstey & Birmingham, 1978; Brown *et al.*, 1979), and in others Mg^{2+}-free solutions were used (Jurkiewicz & Jurkiewicz, 1976; Jenkins *et al.*, 1977; Drew, 1978). As a preliminary to other experiments we have investigated the influence of the presence or absence of Mg^{2+} in the bath medium on agonist responses in the prostatic and epididymal portions of the rat vas deferens (Anton *et al.*, 1977).

Rats (Wistar, 230–270 g) were killed by stunning and exsanguination and bisected vas deferens preparations set up in organ baths (37°C) containing normal Krebs (mM: NaCl 118.1; KCl 4.7; $MgCl_2$ 1.2; KH_2PO_4 1.2; $CaCl_2$ 2.5; $NaHCO_3$ 25.0; glucose 11.1) or Mg^{2+}-free Krebs (normal Krebs minus $MgCl_2$). Responses were measured isometrically and nerve stimulation induced by parallel platinum electrodes (0.1 Hz; 1 ms; supramaximal voltage). The $-\log ED_{50}$ of drugs producing contraction (noradrenaline

Table 1 Sensitivity of various agonists in the bisected vas deferens of the rat in normal and Mg^{2+}-free Krebs

		Normal Krebs		*Mg^{2+}-Free Krebs*	
		$-log\ ED_{50}$ (M)	n	$-log\ ED_{50}$ (M)	n
Noradrenaline	Prostatic	4.28 ± 0.10	10	4.72 ± 0.08*	12
	Epididymal	4.99 ± 0.08	6	5.12 ± 0.12	11
Potassium chloride	Prostatic	2.04 ± 0.05	6	2.17 ± 0.03*	8
	Epididymal	2.06 ± 0.03	6	2.16 ± 0.02*	8
		$-log\ ID_{30}$ (M)	n	$-log\ ID_{30}$ (M)	n
Clonidine	Prostatic	7.96 ± 0.14	10	7.79 ± 0.12	10
	Epididymal	8.48 ± 0.12	9	7.47 ± 0.30*	10
D-ala^2-leu-enkephalin	Prostatic	5.22 ± 0.18	7	5.58 ± 0.15	6
	Epididymal	6.03 ± 0.30	7	4.98 ± 0.18*	6

Values shown are mean ± standard error. *n*—No. of observations.
* – $P < 0.05$ (Students *t* test)—significantly different from normal Krebs.
$-\log ED_{50}$—dose producing 50% of the maximum response.
$-\log ID_{30}$—dose producing 30% inhibition of nerve-induced twitch height.
Responses to potassium chloride were obtained in the presence of 100 μM phentolamine in order to offset effects of released noradrenaline.

and potassium chloride) and the $-\log ID_{30}$ of drugs inhibiting nerve induced twitches (clonidine and D-ala^2-leucine-enkephalin) were calculated and compared in normal and Mg^{2+}-free Krebs.

The initial twitch response to nerve stimulation was similar in normal (prostatic 0.56 ± 0.05 g; epididymal 0.55 ± 0.06 g) and in Mg^{2+}-free Krebs (prostatic 0.70 ± 0.05 g; epididymal 0.50 ± 0.03 g). However, as can be seen from Table 1 the presence or absence of Mg^{2+} in the bath medium modified the drug sensitivity of the vas, although the nature of the effect depended on the portion of vas studied and on whether the drug acted presynaptically or postsynaptically. In particular it seems that some presynaptic elements in the epididymal portion have the property that the effect on them of clonidine and D-ala^2-leucine-enkephalin is Mg^{2+} dependent, and that presynaptic elements in the prostatic portion do not possess this property.

S. Patel is an S.R.C. student.

References

ANSTEY, M.D. & BIRMINGHAM, A.T. (1978). The response of the circular muscle layer of the guinea-pig isolated vas deferens to transmural electrical stimulation. *Br. J. Pharmac.*, **63**, 617–625.

ANTON, P.G., DUNCAN, M.E. & MCGRATH, J.C. (1977). An analysis of the anatomical basis for the mechanical response to motor nerve stimulation of the rat vas deferens. *J. Physiol.*, **273**, 23–43.

BROWN, C.M., MCGRATH, J.C. & SUMMERS, R.J. (1979). The effects of α-adrenoceptor agonists and antagonists on responses of transmurally stimulated prostatic and epididymal portions of the isolated vas deferens of the rat. *Br. J. Pharmac.*, **66**, 553–564.

DREW, G. M. (1978). The effect of different calcium concentrations on the inhibitory effect of presynaptic α-adrenoceptors in the rat deferens. *Br. J. Pharmac.*, **63**, 417–419.

JENKINS, D.A., MARSHALL, I. & NASMYTH, P.A. (1977). An inhibitory role for noradrenaline in the mouse vas deferens. *Br. J. Pharmac.*, **61**, 649–655.

JURKIEWICZ, A. & JURKIEWICZ, N.H. (1976). Dual effect of α-adrenoceptor antagonists in the rat isolated vas deferens. *Br. J. Pharmac.*, **56**, 169–178.

Effects of N^6-2′-O-dibutyryl adenosine 3′,5′-monophosphate on body temperature in the restrained rat

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N^6-2′-O-dibutyryl adenosine 3′,5′-monophosphate (db cAMP) administered centrally can induce hypothermia in the rabbit (Duff, Cranston & Luff, 1972), the cat (Varagić & Beleslin, 1973) and the mouse (Dascombe, Milton, Nyemitei-Addo & Pertwee, in the press), but apparently not in the rat where hyperthermia only is produced (Breckenridge & Lisk, 1969). Breckenridge & Lisk (1969) used 500 μg of solid db cAMP implanted into the anterior hypothalamus of the rat whereas other workers injected i.c.v. solutions containing 4–500 μg db cAMP. The difference between the thermoregulatory effects of db cAMP in various species could be due to differences in drug treatment. We have re-examined the effect of db cAMP on body temperature in the rat.

Rectal and tail skin temperatures were measured in male Wistar rats (275–375 g) held in plastic restraining boxes at a room temperature of 17 ± 1°C (Cox, Kerwin & Lee, 1978). Air temperature inside the boxes was 22 ± 1°C. Db cAMP (sodium salt) dissolved in 1 μl sterile, pyrogen-free 0.9% NaCl was injected into the preoptic and anterior hypothalamic nuclei (PO/AH) through chronically implanted guide cannulae. Temperature responses were assessed as thermal response indices for 4 h (Dascombe & Milton, 1976).

Db cAMP produced a dose-related fall in rectal temperature in most rats with no significant effect on tail skin temperature or behaviour (Table 1). Convulsions were induced in some rats by db cAMP (100 μg or 200 μg) associated with hyperthermia lasting up to 3 h (mean maximum rise in rectal temperature 1.55 ± s.e. mean 0.45°C 1 h after injection of 200 μg db cAMP, $n = 3$). Saline (1 μl) and sodium *n*-butyrate (42.5 μg) injected into the PO/AH caused a rise in rectal temperature lasting more than 4 h in some rats (Table 1). A similar sustained pyrexia was produced by sham injection and was attributable to tissue damage seen at the injection site *post mortem*.

These results show db cAMP can produce hypothermia and hyperthermia in the rat. The mechanism for heat loss caused by db cAMP is unknown, but does not involve heat loss from the tail. Hyperthermia appears to be secondary to convulsive motor activity.

Table 1 Effects of db cAMP on rectal and skin temperatures in the restrained rat

Treatment	Dose	n	Thermal Response Index (mean ± s.e. mean °C.h) Rectal	Tail Skin
Saline	1 µl	5	+2.58 ± 0.98	+0.10 ± 1.44
Sodium *n*-butyrate	42.5 µg	5	+2.27 ± 0.61	+1.83 ± 1.49
Sham injection	—	4	+2.88 ± 0.63	−3.94 ± 2.99
db cAMP	10 µg	5	+2.48 ± 0.76	−3.99 ± 2.46
	50 µg	6	+1.03 ± 1.06	+1.24 ± 3.37
	100 µg	5	+0.09 ± 1.67	−0.90 ± 2.40
	100 µg	1†	+5.33	−6.96
	200 µg	7	−2.29 ± 1.22**	−1.75 ± 0.83
	200 µg	3†	+0.36 ± 1.03	−2.08 ± 1.37

† Rats convulsed.
** Significance of the difference from saline $0.01 > P > 0.001$.

References

BRECKENRIDGE, B.M. & LISK, R.D. (1969). Cyclic adenylate and hypothalamic regulatory functions. *Proc. Soc. exp. Biol. Med.* **131**, 934–935.

COX, B., KERWIN, R. & LEE, T.F. (1978). Dopamine receptors in the central thermoregulatory pathways of the rat. *J. Physiol.*, **282**, 471–483.

DASCOMBE M.J. & MILTON, A.S. (1976). Cyclic adenosine 3',5'-monophosphate in cerebrospinal fluid during thermoregulation and fever. *J. Physiol.* **263**, 441–463.

DASCOMBE, M.J., MILTON, A.S., NYEMITEI-ADDO, I. & PERTWEE, R.G. (in the press). Thermoregulatory effects of N^6-2'-O-dibutyryl adenosine 3',5'-monophosphate in the restrained mouse. *Br. J. Pharmac.*

DUFF, G.W., CRANSTON, W.I. & LUFF, R.H. (1972). Cyclic 3',5'-adenosine monophosphate in central control of body temperature. *Proc. 5th International Congress of Pharmacology*, abstract 360.

VARAGIĆ, V.M. & BELESLIN, D.B. (1973). The effect of N-2-O-dibutyryl-adenosine-3',5'-monophosphate, adenosine triphosphate and butyrate on the body temperature of conscious cats. *Brain Res.*, **57**, 252–254.

Effects of intrahypothalamic injections of adenosine 3',5'-monophosphate and dopamine on thermoregulation in restrained mice

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N^6-2'-O-dibutyryl adenosine 3',5'-monophosphate lowers deep body temperature when injected into the third cerebral ventricles of restrained mice (Dascombe, Milton, Nyemitei-Addo & Pertwee, 1980). Adenosine 3',5'-monophosphate (cyclic AMP) also lowers mouse body temperature when injected intraventricularly (20 and 60 nmol) and on a molar basis is about 10 times less potent than its dibutyryl derivative (Nyemitei-Addo & Pertwee, unpublished). Although it is likely that the hypothermia produced in these experiments resulted principally from an action on hypothalamic thermoregulatory pathways, extrahypothalamic sites of action cannot be excluded. Therefore we have investigated the effect of intrahypothalamic injections of cyclic AMP on thermoregulation. Experiments have also been performed with dopamine since hypothermia produced by intrahypothalamic injections of dopamine in rats may be associated with increased synthesis of cyclic AMP (Cox, Kerwin & Lee, 1978).

Male mice were used at an ambient temperature of 22°C. Cyclic AMP (sodium salt) and dopamine hydrochloride dissolved in a 9 mg/ml NaCl solution (saline) were injected unilaterally (0.5 µl) into the preoptic anterior hypothalamic nuclei of 7 to 10 animals through injection cannulae lowered into previously implanted cannula guides (Nyemitei-Addo,

Pertwee & Tavendale, 1980). Guides were inserted to a depth of 3 mm, 0.5 mm left of the midline and 4.5 mm anterior to the lambda. The tip of each injection cannula extended 1.5 mm beyond that of the guide. Changes in heat production were detected by measurement of oxygen consumption (V_{O_2}) and in heat loss by measurement of paw temperature (Pertwee & Tavendale, 1977). Rectal and paw temperatures were measured with thermistor probes.

Dopamine (53 nmol) produced a maximum fall in rectal temperature of 3.7 ± 0.4°C (mean ± s.e. mean; $P < 0.001$) and also a transient rise in paw temperature (1.0 ± 0.2°C; $P < 0.001$) and a fall in V_{O_2}. Between 2 and 12 min after injection V_{O_2} was 63 ± 6 ml 25 g^{-1} h^{-1} ($P < 0.001$) below its preinjection value. Lower doses of dopamine, 0.53 and 5.3 nmol, also produced significant falls in body temperature. These were respectively 0.7 ± 0.2 and 1.3 ± 0.2°C. Saline had no detectable effect on thermoregulation. Like dopamine, cyclic AMP (60 nmol) produced marked hypothermia (2.6 ± 0.3°C; $P < 0.001$). This was associated with falls in V_{O_2}, 62 ± 7 ml 25 g^{-1} h^{-1} ($P < 0.001$) between 2 and 12 min after injection, and in 5/7 mice with transient rises in paw temperature. The hypothermic effects of intrahypothalamic injections of dopamine (6.6 nmol) and cyclic AMP (33 nmol) were significantly reduced in animals pretreated 30 min before with pimozide (0.5 or 2.5 mg/kg i.v.).

These results support the hypothesis that in mice kept at an ambient temperature below thermal neutrality, dopamine and cyclic AMP can each reduce deep body temperature by acting on hypothalamic thermoregulatory pathways to lower heat production and increase heat loss. The question of whether cyclic AMP was acting extracellularly or intracellularly to alter thermoregulation and the nature of the mechanism underlying the interaction between pimozide and cyclic AMP are both aspects of this study requiring further investigation.

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References

COX, B., KERWIN, R. & LEE, T.F. (1978). Dopamine receptors in the central thermoregulatory pathways of the rat. *J. Physiol.*, **282**, 471–483.

DASCOMBE, M.J., MILTON, A.S., NYEMITEI-ADDO, I. & PERTWEE, R.G. (1980). Thermoregulatory effects of N^6-2′-O-dibutyryl adenosine 3′,5′-monophosphate in the restrained mouse. *Br. J. Pharmac.* In press.

NYEMITEI-ADDO, I., PERTWEE, R.G. & TAVENDALE, R. (1980). Stereotaxic implantation of cannulae for subsequent drug administration into the third ventricles of conscious mice. *Br. J. Pharmac.*, **68**, 189P.

PERTWEE, R.G. & TAVENDALE, R. (1977). Effects of Δ^9-tetrahydrocannabinol on the rates of oxygen consumption of mice. *Br. J. Pharmac.*, **60**, 559–568.

Dopamine-β-hydroxylase activity in cerebrospinal fluid and serum of rabbits after electroconvulsive shock

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The effect of electroconvulsive shock on the noradrenergic system has been the subject of extensive studies summarized by Grahame-Smith, Green & Costain in 1978. In the present study we investigated the effect of electroconvulsive shock treatment on the release of central and peripheral noradrenaline.

Two groups of rabbits were subjected to electroconvulsive shock resulting in overt convulsions, the first group to a single shock, the second group to repeated shocks (once daily for ten days). The effect of this stimulus on the release of the central and peripheral noradrenergic neurotransmitter was evaluated on the basis of estimations of the dopamine-β-hydroxylase activity in the cerebrospinal fluid (CSF) and in the serum, respectively (De Potter, Pham-Huu-Chanh, De Smet & De Schaepdryver, 1976).

Application of a single electroshock as well as of daily repeated electroshocks was not followed by significant change in CSF-DβH activity but resulted in a 3- to 4-fold increase in serum-DβH activity 3 to 5 h after electroshock, which returned to control values 12 to 16 h after electroshock. Electroconvulsive shock in rabbits pretreated with 6-hydroxydopamine (500 μg intracerebroventricularly, twice with a one-week interval) was not followed by any change in CSF-DβH activity in the first 6 h after electroshock, but did again result in a 3- to 4-fold increase in serum DβH activity 3 to 4 h after electroshock.

These observations suggest that the release of the central noradrenergic neurotransmitter is not primarily involved in both the central (Grahame-Smith, Green & Costain, 1978) and peripheral (De Schaepdryver & Piette, 1961) effects of electroconvulsive shock.

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References

DE POTTER, W.P., PHAM-HUU-CHANH, C., DE SMET, F. & DE SCHAEPDRYVER, A.F. (1976). The presence of dopamine-β-hydroxylase in the cerebrospinal fluid of rabbits and its increased concentration after stimulation of peripheral nerves and cold stress. *Neuroscience*, **1**, 523–529.

DE SCHAEPDRYVER, A.F. & PIETTE, Y. (1961). Electroshock and tissue catecholamine content in reserpinized rabbits. *Arch. int. Pharmacodyn.*, **130**, 225–230.

GRAHAME-SMITH, D.G., GREEN, A.R. & COSTAIN, D.W. (1978). Mechanism of the antidepressant action of electroconvulsive therapy. *The Lancet*, **Febr. 4**, 254–257.

A dissociation of dopamine agonist and antagonist binding sites in the rat striatum

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It has been shown that dopamine agonists and antagonists have high affinities for sites labelled with agonist and neuroleptic ligands respectively. In the two state hypothesis it is suggested that agonists and antagonists label different states of the same receptor (Burt, Creese & Snyder, 1976), but it is also possible that agonists and antagonists label two distinct receptors (Titeler & Seeman, 1978). In the present studies we attempt a dissociation of dopamine agonist and antagonist binding sites using the agonist ligand [^{3}H]-N-n-propylnorapomorphine [^{3}H]-NPA (Creese, Padgett, Fazzini & Lopez, 1979; Titeler & Seeman, 1979), the neuroleptic ligand [^{3}H]-spiperone, and a novel aporphine derivative shown to irreversibly inactivate the dopamine 'agonist' receptor, (−)N-(2-chloroethyl)norapomorphine {(−)NCA} (Costall, Fortune, Law, Naylor, Neumeyer & Nohria, 1980; Law, Neumeyer, Kula & Baldessarini, 1980).

Rat striatal tissue was homogenized in 15 mM Tris–HCl buffer (pH 7.6, 25°C), incubated for 10 min at 37°C, centrifuged and resuspended twice, and finally resuspended in Tris–HCl buffer containing ascorbic acid (0.01%), nialamide (12.5 μM) and Na_2EDTA (1.0 mM) [^{3}H]-NPA (80.2–56.2 Ci/mmol) and [^{3}H]-spiperone (20.0 Ci/mmol) were incubated for 15 min at 25°C, each assay tube containing 5 mg tissue. Samples were rapidly filtered under vacuum and washed, and bound radioactivity determined at 45% efficiency. Specific binding was defined as the difference between tritiated ligand binding in the absence and presence of 2-amino-6,7-dihydroxytetralin (10 μM, ADTN). The presence of Na_2EDTA was found to be essential for satisfactory binding of [^{3}H]-NPA, thus, whilst the total specific binding of [^{3}H]-NPA in the absence of this agent was considerably higher, this binding lacked stereospecificity as defined using (+) and (−) butaclamol, and very high concentrations ($>10^{-7}$ M) of non-labelled (−)NPA, apomorphine and ADTN were required to displace the [^{3}H]-NPA. 0.125–2.0 nM [^{3}H]-NPA and [^{3}H]-spiperone caused saturable binding which was displaced by nM concentrations of the respective non-labelled compounds, or (+) butaclamol. A 15 min preincubation with (−)NCA (10^{-9}–10^{-5} M) antagonized the binding of 0.25 nM [^{3}H]-NPA (IC_{50} 17 nM); higher concentrations of (−)NCA (10^{-5}–10^{-4}) were required to antagonize the binding of 0.25 nM [^{3}H]-spiperone. A preincubation with 25 μM (−)NCA abolished the binding of 0.125–1.5 nM [^{3}H]-NPA, but only reduced the binding of lower concentrations of [^{3}H]-spiperone, failing to modify the binding of concentrations >1.0 nM. The inhibition of binding of 0.25 nM [^{3}H]-NPA caused by 25 μM (−)NCA could not be reversed by repeated washing of the membranes.

The essential finding of the present study is the ability of (−)NCA to differentially antagonize the binding of [^{3}H]-NPA and [^{3}H]-spiperone. If it is accepted that [^{3}H]-NPA labels a 'dopamine agonist receptor', that [^{3}H]-spiperone labels a 'dopamine antagonist receptor', and that (−)NCA acts preferentially on the agonist receptor in such an irreversible or persistent manner as to preclude the possibility of interconversion of 'agonist' to 'antagonist' state, then the selective inhibition of [^{3}H]-NPA binding by (−)NCA would support the hypothesis of distinct receptor entities to allow dopamine agonist and antagonist binding.

References

BURT, D.R., CREESE, I. & SNYDER, S.H. (1976). Properties of [^{3}H]-haloperidol and [^{3}H]-dopamine binding associated with dopamine receptors in the calf brain membranes. *Mol. Pharmac.*, **12**, 800–812.

COSTALL, B., FORTUNE, D.H., LAW, S.-J., NAYLOR, R.J., NEUMEYER, J.L. & NOHRIA, V. (1980). (−)N-(Chloroethyl)norapomorphine inhibits striatal dopamine function via irreversible receptor binding. *Nature*, in press.

CREESE, I., PADGETT, L., FAZZINI, E. & LOPEZ, F. (1979). [^{3}H]-N-n-propylnorapomorphine: a novel agonist ligand for central dopamine receptors. *Eur. J. Pharmac.*, **56**, 411–412.

LAW, S.-J., NEUMEYER, J.L., KULA, N. & BALDESSARINI, R.J. (1980). (−)N-(Chloroethyl)-10,11-dihydroxynorapomorphine (Chloroethylnorapomorphine), a novel irreversible dopamine receptor antagonist. *J. Med. Chem.*, in press.

TITELER, M. & SEEMAN, P. (1978). Antiparkinsonian drug doses and neuroleptic receptors. *Experientia*, **34**, 1490–1492.

TITELER, M. & SEEMAN, P. (1979). Selective labelling of different dopamine receptors by a new agonist [^{3}H]-Ligand: [^{3}H]-N-propylnorapomorphine. *Eur. J. Pharmac.*, **56**, 291–292.

Continuous administration of trifluoperazine to rats for six months only alters cerebral dopamine receptor characteristics

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The continuous administration in drinking water of trifluoperazine dihydrochloride to rats for six months or longer results in a reversal of the initial dopamine receptor blockade such that the animals exhibit enhanced stereotyped behaviour and increased [^{3}H]-spiperone binding sites while still receiving daily drug intake (Glow, Theodorou, Jenner & Marsden, 1979; Clow, Jenner & Marsden, 1979). Neuroleptic drugs, such as trifluoperazine, have direct and indirect actions on a number of cerebral transmitter systems other than the dopamine systems. In the present study we have investigated whether the chronic administration of trifluoperazine dihydrochloride to rats alters ligand binding to a range of brain transmitter receptors.

Male Wistar rats (180 ± 4 g at the start of the study) received trifluoperazine dihydrochloride (3.0–5.0 mg/kg/day) in drinking water over a six month period. Age-matched control animals were maintained on normal drinking water alongside the drug treated group. After the six month period the animals receiving trifluoperazine exhibited an enhanced stereotyped response to the administration of apomorphine hydrochloride (0.5 mg/kg s.c. 15 min previously) when compared to control animals. At this time animals from control and drug-treated groups were killed and the brains rapidly dissected to obtain frontal cortex, remaining cortex, cerebellum, striatum and mesolimbic (nucleus accumbens plus tuberculum olfactorium) tissue pools, which were then frozen at −70°C until analysis. All radioactive ligands were used in a range of concentrations to obtain Scatchard plots from which the number of binding sites (B_{max}) and the dissociation constant (K_D) were calculated.

Specific binding of [^{3}H]-spiperone (0.1–1.0 nM) to dopamine receptors, as judged using 1000-fold excess of (+)-butaclamol for striatal and mesolimbic tissue and in addition using a 100-fold excess of the 5-HT antagonist R43448 in all mesolimbic samples showed K_D to be increased in the striatal and mesolimbic tissue from drug treated animals. An increase in B_{max} was only apparent in striatal preparations. In frontal cortex specific binding of [^{3}H]-spiperone (0.2–40 nM; as judged using a 1000-fold excess of (+)-butaclamol) to 5-HT receptors showed K_D to be again increased but no change in B_{max} was apparent.

In striatal, mesolimbic and total cortex preparations the specific binding of [^{3}H]-dexetimide (0.3–4.0 nM; as judged using 100-fold excess dexetimide) revealed no change in B_{max} or K_D for cholinergic receptors in any region. Similarly, the specific binding of [^{3}H]-mepyramine (0.2–4.0 nM; as judged using 1000-fold excess astemizole) or [^{3}H]-WB410/ (0.1–2.0 nM; as judged using 5000-fold excess noradrenaline) to total cortex histamine and noradrenaline receptors respectively also showed no change in the kinetic constants K_D and B_{max}. In addition, no change in K_D or B_{max} for the specific binding of [^{3}H]-muscimol (0.2–4.0 nM; as judged using 2.2×10^{-5} M GABA) to cerebellar GABA receptors was found.

The results suggest that the increase in dopamine receptor function caused by the continuous long-term administration of trifluoperazine may be specific for dopamine and does not represent a general change in brain receptor function.

References

CLOW, A., JENNER, P. & MARSDEN, C.D. (1979). Changes in dopamine-mediated behaviour during one years neuroleptic administration. *Eur. J. Pharmac.*, **57**, 365–375.

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.

Behavioural changes induced by the unilateral intrastriatal administration of glutamate analogues in the rat

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The unilateral intrastriatal injection of kainic acid causes circling in rodents that is dependent upon the presence of intact cortico-striatal glutamate fibres (Jenner, Marsden, Reavill & Taylor, 1980). In the present study we have investigated the ability of a range of glutamate analogues with potent neuro-excitatory actions to mimic the effects of kainic acid injection into the striatum. In addition, we have examined the ability of some excitatory amino-acid antagonists to inhibit kainic acid-induced circling.

Unilateral intrastriatal injection of kainic acid (0.5–5.0 μg in 1 μl 0.9% saline) into female Cobs Wistar rats (150–200 g) caused dose-related circling. A consistently effective dose of kainic acid (2 μg) caused three phases of circling. Initial weak ipsiversive rotation was followed by marked contraversive rotation, which, after 10 to 24 h was succeeded by weak inconsistent ipsiversive circling.

Domoic acid (0.5–10 μg in 1 μl 0.9% saline) also caused dose-related circling similar to that produced by kainic acid, characterised by initial ipsiversive rotation followed by contraversive rotation. N-methyl-D-aspartic acid (2 or 5 μg in 1 μl 0.9% saline) and ibotenic acid (20–100 μg in 1 μl 0.9% saline) induced initial ipsiversive rotation but no subsequent contraversive rotation was observed. Quisqualic acid (1–20 μg in 1 μl 0.9% saline) and α-allokainic acid (1–10 μg in 1 μl 0.9% saline) did not produce rotation. Higher doses of α-allokainic acid (20–100 μg in 1 μl 0.9% saline) produced contraversive circling without initial ipsiversive rotation.

Kainic acid (0.5–5.0 μg), domoic acid (0.5–10 μg), N-methyl-D-aspartic acid (5 or 10 μg), ibotenic acid (20–100 μg) and α-allokainic acid (20–100 μg) induced seizure activity in the form of repeated rolling or rearing paroxysmal fits.

All compounds were neutralized with 2 N NaOH prior to injection (final pH of solution 7.0) but injection of the appropriate vehicle produced none of these behavioural effects.

Glutamic acid dimethylester (250 μg in 1 μl 0.9% saline), 2-amino-4-phosphonobutyric acid (50 μg in 1 μl 0.9% saline), DL-α-methylglutamic acid (20 μg in 1 μl 0.9% saline) injected into one striatum in the same solution as kainic acid did not modify kainic acid induced circling behaviour over the subsequent 10 h observation period. In addition the administration of glutamic acid diethylester (50 or 250 μg in 1 μl 0.9% saline) or HA 966 (23 μg in 1 μl 0.9% saline adjusted to pH 6–7 with 1 N HCl) 30 min prior to kainic acid administration again did not modify the circling response to kainic acid. Further, direct intrastriatal injection of γ-D-glutamylglycine (102 μg in 0.5 μl 0.9% saline containing 1 equivalent 2 N NaOH) or a combination of (±)-2-amino-5-phosphonovaleric acid (19.7 μg) and (±)-cis-2,3-piperidine dicarboxylic acid (86.6 μg) in the same solution (0.5 μl 0.9% saline containing two equivalents 2 N NaOH) 1 min prior to kainic acid also did not modify the kainate-induced circling behaviour. None of the antagonists caused rotational behaviour when injected alone.

The data show no close correlation with the actions of these compounds as demonstrated by iontophoretic electrophysiological studies (Watkins, personal communication). Some of the differences may reflect actions on kainate as against glutamate receptors, for glutamic acid itself does not mimic the effect of kainic acid when injected into the striatum; or, as receptor studies suggest, different actions on kainate, N-methyl-D-aspartic acid or quisqualate receptors (Watkins, personal communication). However, other factors are probably involved and may include solubility, rate of diffusion and degradation.

Reference

JENNER, P., MARSDEN, C.D. & TAYLOR, R.J. Kainate-induced circling behaviour in rats. *Br. J. Pharmacol.*, (in press).

Method for determining the CNS penetration of enkephalin analogues in the rat

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A method in the rat suitable for comparing the extent to which opioid peptides penetrate the central nervous system after peripheral administration has been developed.

Male rats (250–300 g) were anaesthetized with sodium hexobarbital (100 mg/kg i.p.) for a period of 30–45 minutes. The right jugular vein was cannulated so that the tip of the cannula was at the entrance of the right atrium and the free end of the cannula led out through a puncture wound in the skin at the side of the neck. With the rat lying prone, the skin in the neck was cut along the mid-line, the underlying muscles separated with forceps and the gap enlarged until the cisterna magna became visible. The muscle attachments to the back of the skull and the atlas vertebra were separated from the bone with a scalpel and the muscle over the cisterna magna was removed without damaging the cisterna magna membrane. The shoulders of the rat were raised slightly and its head tilted downward to increase and flatten the exposed membrane. After the membrane had been gently dried with a warm air fan, a bevelled metal cannula (21 gauge) 4 mm long and attached at the non-bevelled end to a polythene cannula, was stabbed through the membrane to a depth of 2 mm and held in position by a layer of contact adhesive (alpha-cyanoacrylate, 3 M, Zürich) smeared on the lower side of a plastic guard ring (2 mm diameter) glued round the metal cannula 2 mm from the point. The glue was allowed to dry for 2 min, before the skin over the neck would was loosely sewn together. The presence of cerebrospinal fluid (CSF) in the cannula was taken as evidence of a successful operation. Four hours later when the rats were conscious, 50 µl samples of CSF were removed with the aid of an infusion pump over 5 min periods, 30 min and immediately before and immediately after the i.v. infusion of the peptide over a 30 min period. Venous blood samples were taken simultaneously and the amounts (ng/ml) of the peptide in both the serum and CSF samples were measured using antibodies raised in rabbits against FK 33-824 (sensitivity limit 40 pg/ml). Morphine was measured with a RIA kit (Abuscreen® Roche) modified to give a sensitivity of 400 pg/ml.

The results (Table 1) showed that only very low amounts of the metabolically stable enkephalin analogues FK 33-824 and FW 34-569 penetrated the CSF, thus offering an explanation for the marked difference in the antinociceptive potencies of the peptides observed after intracerebroventricular (i.c.v.) and after i.v. administration (Pless *et al.*, 1977).

Reference

PLESS, J., BAUER, W., CARDINAUX, F., CLOSSE, A., HAUSER, D., HUGUENIN, R., RÖMER, D., BÜSCHER, H. & HILL, R. C. In *Molecular Immunology*, Edit. MacIntyre and Szelke, Elsevier/North-Holland Biomedical Press, 1977.

Table 1 Comparison of the serum and CSF levels of 2 enkephalin analogues after i.v. infusion over 30 min (0.5 mg/kg, 30 min.) in the conscious rat

Test Compound (n = 4)	*Serum*	*CSF* (ng/ml)	*CSF/Serum* (× 100)
Tyr-DAla-Gly-MePhe-Met(O)ol (FK 33-824)	274	9.7	3.5
MeTyr-DAla-Gly-MePhe-Met(O)ol (FW 34-569)	689	20.3	2.9
Morphine HCl	140	29	21

The effects of zimelidine on changes in brain 5-hydroxyindoles and plasma corticosterone following chronic treatment with morphine

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Both the acute administration of opiates and their withdrawal have been shown to increase plasma corticosterone levels in the rat (Kokka, Garcia & Elliott, 1973; Eisenberg & Sparber, 1979). Similar changes in plasma corticosterone secretion are seen in response to nicotine and have been related tentatively to changes in 5-hydroxytryptamine (5-HT) secretion in the hippocampal formation (Benwell & Balfour, 1979). The purpose of the present study was to use zimelidine, a selective inhibitor of the neuronal 5-HT reuptake mechanism (Ross, Ogren & Renyi, 1976), to investigate the possible role of 5-HT systems in the effects of morphine on plasma corticosterone.

Male Sprague–Dawley rats (Charles River) were given subcutaneous injections (3 × daily) of morphine or saline for 15 days. Increasing doses of morphine were administered over the first 9 days according to the schedule of Gianutos, Hynes, Puri, Drawbaugh & Lal (1974), a schedule which has been shown to induce physical dependence consistently in rats. 40 h before the end of the experiment half the morphine-treated rats were withdrawn and given saline in place of morphine. Zimelidine (30 mg/kg i.p.) was given to half the rats in each group (morphine-treated, morphine-withdrawn and the controls) 16 h and 2 h before the animals were killed by cervical dislocation. Blood samples were taken from the trunks and assayed for plasma corticosterone (Mattingly, 1962) and the brains were removed, dissected into the hypothalamus, hippocampal formation and remainder and assayed for 5-HT and 5-hydroxyindole acetic acid (5-HIAA) using the method of Curzon & Green (1970).

Morphine administration increased corticosterone from 9 ± 1 μg/100 ml to 23 ± 2 μg/100 ml ($P < 0.01$), residual brain 5-HT from 0.145 ± 0.007 μg/g to 0.185 ± 0.010 μg/g ($P < 0.05$) and residual brain 5-HIAA from 0.176 ± 0.012 μg/g to 0.215 ± 0.022 μg/g ($P < 0.05$). However the brain 5-hydroxyindole concentrations did not remain elevated when morphine was withdrawn in spite of the fact that plasma corticosterone was increased to 34 ± 8 μg/100 ml in this group. No changes in 5-hydroxyindole levels were observed in either of the other brain regions studied.

Zimelidine blocked the effects of morphine administration on both plasma corticosterone and residual brain 5-hydroxyindole levels. It also significantly reduced ($P < 0.05$) the increased plasma corticosterone levels observed in the withdrawn rats. In addition zimelidine caused consistent and significant falls in 5-HIAA in all the brain regions studied in both control and morphine-treated rats. However these reductions in 5-HIAA were not observed if the rats had been withdrawn from morphine.

The data provde some evidence to suggest that the effects of morphine on plasma corticosterone may be mediated through brain 5-HT systems but do not implicate the hypothalamus or hippocampus specifically.

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References

BENWELL, M.E.M. & BALFOUR, D.J.K. (1979). Effects of nicotine and its withdrawal on plasma corticosterone and brain 5-hydroxyindoles. *Psychopharmacology*, **63**, 7–11.

CURZON, G. & GREEN, A.R. (1976). Rapid method for the determination of 5-hydroxytryptamine and 5-hydroxyindole acetic acid in small regions of rat brain. *Br. J. Pharmac.*, **39**, 653–655.

EISENBERG, R.M. & SPARBER, S.B. (1979). Changes in plasma corticosterone levels as a measure of acute dependence upon levorphanol in rats. *J. Pharmac. Exp. Ther.*, **211**, 364–469.

GIANUTOS, G., HYNES, M.D., PURI, S.K., DRAWBAUGH, R.B. & LAL, H. (1974). Effect of apomorphine and nigrostriatal lesions on aggression and striatal dopamine turnover during morphine withdrawal: evidence for dopamine super-sensitivity in protracted abstinence. *Psychopharmacology*, **34**, 37–44.

KOKKA, N., GARCIA, J.F. & ELLIOTT, H.W. (1973). Effects of acute and chronic administration of narcotic analgesics on growth hormone and corticotrophin (ACTH) secretion in rats. *Proc. Brain Res.*, **39**, 347–360.

MATTINGLY, D. (1962). A simple fluorometric procedure for the estimation of 11-hydroxycorticosteroids in human plasma. *J. Clin. Path.*, **15**, 374–379.

ROSS, S.B., OGREN, S.O. & RENYI, A.L. (1976). (2)-Dimethyl-amino-1-(4-bromophenyl)-1-(3-pyridyl) propene (H102/09), a new selective inhibitor of neuronal 5-hydroxytryptamine uptake. *Acta pharmac. toxicol.*, **39**, 152–166.

Rebound decrease in CNS excitability following a single dose of amphetamine

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We have demonstrated that acute administration of high doses, or chronic administration of low doses, of CNS depressant drugs leads to a withdrawal hyperexcitability of the CNS in the rat (Turnbull & Watkins, 1976a; Turnbull & Watkins, 1976b). We have measured the change in excitability of the CNS by determining the sensitivity of the animals to the anaesthetic halothane. This method has the advantage that the same animals can be tested at intervals (Turnbull & Watkins, 1976a). The same withdrawal hyperexcitability can be demonstrated, however, by other methods e.g. decreased sensitivity to chemical convulsant drugs (Turnbull, Watkins & Wheeler, 1980) or increased tendency to audiogenic seizures (Crossland & Turnbull, 1972). In the present experiments we have determined whether a withdrawal decrease in CNS excitability is apparent following administration of stimulant drugs.

The first experiments were performed with rats treated chronically with bemegride. Groups of eight female Wistar rats, weighing approximately 100 g, were given a solution of bemegride (0.2 mg/ml) containing saccharin (0.125 mg/ml) as their sole drinking fluid for 4 days, 1 week or 2 weeks. The mean daily dose for each group was as follows: 4 days, 15 mg/kg/day; 1 week, 15 mg/kg/day; 2 weeks, 18.8 mg/kg/day. Halothane-induced sleeping time was measured at the time of substitution of the drug solution by tap water and at intervals thereafter until the sensitivity to halothane returned to normal (results expressed as mean ± s.e. mean % of control). Immediately after drug substitution the halothane sleeping time did not differ from that of control animals. Animals treated with bemegride for 2 weeks were more sensitive to halothane at 6 h (123 ± 5%) and 20 h (116 ± 5%) after withdrawal; animals treated for 1 week were more sensitive to halothane at 6 h only (116 ± 5%) but animals treated for 4 days never differed significantly from control.

We next investigated the effect of a single large dose of amphetamine sulphate (10 mg/kg i.p.). Halothane sleeping time was monitored at intervals, beginning 1 h after the injection. A marked decrease in sensitivity to halothane was found, peaking at 10 h after the amphetamine injection with the sleeping time being 225 ± 4(6)% of control. The sensitivity had returned to the control level by 24 hours.

We have thus demonstrated the occurrence of a rebound decrease in CNS excitability, as indicated by an increase in sensitivity to the anaesthetic halothane, following administration of CNS stimulant drugs.

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References

CROSSLAND, J. & TURNBULL, M.J. (1972). Gamma-aminobutyric acid and the barbiturate abstinence syndrome in rats. *Neuropharmac.*, **11**, 733–738.

TURNBULL, M.J. & WATKINS, J.W. (1976a). Determination of halothane-induced sleeping time in the rat: effect of prior administration of centrally active drugs. *Br. J. Pharmac.*, **58**, 27–35.

TURNBULL, M.J. & WATKINS, J.W. (1976b). Change in sensitivity to halothane in the rat following administration of sodium barbitone in the drinking water. *Br. J. Pharmac.*, **58**, 285–286P.

TURNBULL, M.J., WATKINS, J.W. & WHEELER, H. (1980). See Proceedings of the Aberdeen meeting of the British Pharmacological Society, September.

The pharmacological spectrum of binodaline, a novel antidepressant drug

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Binodaline, 1-(ω-dimethylaminoethylmethyl)-amino-3-phenyl-indole-hydrochloride, is a novel drug developed in our laboratories (Schatz, Jahn, Wagner-Jauregg, Zirngibl & Thiele, 1980), which shows interesting antidepressant activity in laboratory animals.

Mice and rats were dosed with either binodaline or imipramine and comparisons of pharmacological activity were determined by means of ED_{50} or LD_{50} values (Litchfield & Wilcoxon, 1949): the values in brackets are the 95% confidence limits.

In experiments designed to detect potential antidepressant drugs, binodaline was of similar activity to

Table 1 The effects of binodaline in rats and mice

Test (Reference)	*ED_{50} (95% confidence limits) mg/kg p.o.* *Binodaline*	*Imipramine*	*P*
Antagonism of tetrabenazine-induced ptosis in rats (1)	8.0 (3.3–27.2)	9.0 (3.5–23.4)	>0.05
Inhibition of oxotremorine-induced tremor in mice (2)	>200	20 (3.8–106)	<0.05
	LD_{50} (mg/kg p.o.)		
Mice		400	
Male mice	760 (598–965)		
Female mice	770 (592–1001)		
Rats		625	
Male rats	1380 (1007–1891)		
Female rats	1160 (943–1427)		

ED_{50} and LD_{50} values (with 95% confidence limits) were determined by the method of Litchfield & Wilcoxon (1949). The references for the test methods are as follows: 1. Giurgea, Dauby, Levis & Giurgea (1963); 2. Leslie & Maxwell (1964). The LD_{50} values for imipramine are included for comparative purposes and were taken from Domenjoz & Theobald (1959).

imipramine in antagonizing tetrabenazine-induced ptosis in rats (Table 1). Unlike imipramine, binodaline was ineffective in antagonizing oxotremorine-induced tremor in mice, a test that detects anti-muscarinic activity in the central nervous system (Table 1). This lack of an atropine-like action was confirmed on guinea-pig isolated ileum (concentration for 50% reduction of acetylcholine-induced contractions: atropine 1.2 ± 0.17 nM, $n = 4$; imipramine 0.68 ± 0.11 μM, $n = 4$; binodaline >3.03 μM, $n = 5$; mean ± s.e. mean).

These results show that binodaline is less toxic than imipramine and has similar or greater antidepressant activity in acute experiments in laboratory animals. Furthermore, the most pronounced difference between binodaline and imipramine is that the new drug is devoid of cholinolytic activity.

References

DOMENJOZ, R. & THEOBALD, W. (1959). Zur pharmakologie des tofranil (N-(3-dimethylaminopropyl)-iminodibenzyl-hydrochlorid). *Arch int. Pharmacodyn. Thér.*, **120**, 450–489.

GIURGEA, M., DAUBY, J., LEVIS, S. & GIURGEA, C. (1963). Un test antitetrabenazine modifie, pour le screening des produits antidepressifs. *Med. Exp.*, **9**, 249–262.

LESLIE, G.B. & MAXWELL, D.R. (1964). Inhibition of tremorine by a group of non-anti-Parkinson phenothiazine derivatives. *Nature, Lond.*, **202**, 97–98.

LITCHFIELD, J.T. & WILCOXON, F. (1949). A simplified method of evaluating dose–effect experiments. *J. Pharmac. exp. Ther.*, **96**, 99–113.

SCHATZ, F., JAHN, U., WAGNER-JAUREGG, TH., ZIRNGIBL, L. & THIELE, K. (1980). 1-Amino-3-phenylindoles with antidepressant activity: binodaline hydrochloride and related substances. *Arzneim.-Forsch. (Drug Res.)*, in the press.

Comparison of the analgesic effects of subcutaneous and intracerebroventricular injection of calcitonin on acetic acid-induced abdominal constrictions in the mouse

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Calcitonin possesses analgesic activity after administration by injection into the lateral ventricles of the rabbit (Pecile, Ferri Braga & Olgiati, 1975) by an effect independent of the opiate receptor (Braga, Ferri, Santagostino, Olgiati & Pecile, 1978; Yamamoto, Kumagai, Tachikawa & Maeno, 1979). However, calcitonin also possesses peripheral anti-inflammatory activity (Reisterer & Jaques, 1969; Abdullahi, Bastiani, Nogarin & Velo, 1975; Strettle, Bates & Buckley, 1980) and inhibits the synthesis of prostaglandins and thromboxane (Cesarini, Colombo, Olgiati & Pecile, 1979).

We have investigated the possibility that the analgesic effect of centrally administered calcitonin may be due to a peripheral action.

Acetic acid in 0.154 mol/l NaCl was injected into the peritoneal cavity of CPLA mice (♂ or ♀, 25–45 g BW) (Collier, Dineen, Johnson & Schneider, 1968) and the number of abdominal constrictions was counted between the 10th–14th min inclusive, after the injection of acid.

Salmon calcitonin was administered by subcutaneous or by intracerebroventricular (icv) injection. Calcitonin, dissolved in a solution containing 0.154 mol/l NaCl and 1 g/l bovine serum albumin (BSA), was given by subcutaneous injection either 10 or 20 min prior to the injection of acetic acid. Calcitonin, dissolved in 10 µl of a solution containing 0.100 mol/l NaCl plus 1 g/l BSA and 50 mmol/l Tris buffer pH 7.35, was injected into the ventricle by the method of Haley & McCormick (1957) 10 min prior to the injection of acetic acid. Aspirin suspended in 0.154 mol/l NaCl plus 100 g/l gum acacia was injected s.c. 30 min prior to acetic acid. Control animals received the appropriate vehicle.

Abdominal constrictions began 5–8 min after the injection of acetic acid and the frequency of constrictions was proportional to the concentration of acid (0.25–2.4% v/v) between the 10th–14th min after the injection. 1–1.2% acetic acid was selected for further work. Aspirin (0.125–1.0 mmol/kg) caused a dose related inhibition of the abdominal constrictions with an ED_{50} of 0.44 mmol/kg.

Salmon calcitonin (0.01–1000 MRC u/kg, 20 min prior to acid or 10–50 MRC u/kg 10 min prior to acid) by subcutaneous injection did not affect the response to acetic acid. Subcutaneous injection of 10 MRC u/kg SCT caused a significant fall of 0.46 $mmol.dm^{-3}$ ($P < 0.005$) in the plasma calcium concentration. Salmon calcitonin at doses of 0.040, 0.20, 2.0, 10 and 50 MRC u/kg by icv injection caused 14 ± 6.4, 37 ± 9.3*, 38 ± 15.7*, 50 ± 8.0*, and 31 ± 8.2* (mean ± s.e. mean)% inhibition in the frequency of abdominal constrictions (* = $P < 0.005$; control group $n = 26$, test groups $n = 10$). SCT 10 MRC u/kg by icv injection caused a fall of 0.18 mmol/l in the plasma calcium concentration ($P < 0.05$).

In conclusion, SCT by subcutaneous injection does not produce analgesia. SCT by icv injection produced a potent analgesic effect by a central action upon cells accessible to diffusion from the ventricular system. Salmon calcitonin, after subcutaneous injection, does not readily cross the blood brain barrier.

Salmon calcitonin was donated by J.W. Bastian and J.P. Aldred, Armour Pharmaceutical, Ill., U.S.A.

References

ABDULLAHI, S.E., DE BASTIANI, G., NOGARIN, L. & VELO, G.P. (1975). Effect of calcitonin on carageenan foot oedema. *Agents and actions*, **5**, 371–373.

BRAGA, P., FERRI, S., SANTAGOSTINO, A., OLGIATI, V.R. & PECILE, A. (1978). Lack of opiate receptor involvement in centrally induced calcitonin analgesia. *Life Sci.*, **22**, 971–978.

CESARINI, R., COLOMBO, M., OLGIATI, V.R. & PECILE, A. (1979). Calcitonin and prostaglandin system. *Life Sci.*, **25**, 1851–1856.

COLLIER, H.O.J., DINEEN, L.C., JOHNSON, C.A. & SCHNEIDER, C. (1968). The abdominal constriction response and its suppression by analgesic drugs in the mouse. *Br. J. Pharmacol.*, **32**, 295–310.

HALEY, T.J. & MCCORMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *Brit. J. Pharmacol.*, **12**, 12–15.

PECILE, A., PERRI, S., BRAGA, P.C. & OLGIATI, V.R. (1975). Effects of intracerebroventricular calcitonin in the conscious rabbit. *Experientia*, **31**, 332–333.

REISTERER, L. & JAQUES, R. (1969). Reduction of increased vascular permeability by calcitonin. *Pharmacol.*, **3**, 53.

STRETTLE, R.J., BATES, R.F.L. & BUCKLEY, G.A. (1980). Evidence for a direct anti-inflammatory action of calcitonin; inhibition of histamine-induced mouse pinnal oedema by porcine calcitonin. *J. Pharm. Pharmacol.*, **32**, 192–195.

YAMAMOTO, M., KUMAGAI, F., TACHIKAWA, S. & MAENO, H. (1979). Lack of effect of Levallorphan on analgesia induced by intraventricular application or porcine calcitonin in the mouse. *Europ. J. Pharmacol.*, **55**, 211–213.

Lithium and glucose utilization in human erythrocytes

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Lithium at pharmacological concentrations inhibits a number of magnesium dependent enzymes of glycolysis including phosphofructokinase, enolase, phosphoglycerate kinase (Kajda & Birch, unpublished) and pyruvate kinase (Kajda, Birch, O'Brien & Hullin, 1979). Since glycolysis is the major energy supply of the brain it is clearly important to establish whether or not such inhibition has any relevance to the successful use of lithium in the treatment of recurrent affective psychoses (Birch, 1978). The human erythrocyte utilizes glucose almost exclusively via the glycolytic pathway and for this reason was chosen in the present study to investigate the effects of lithium. The method used is based on that of Bashan, Moses & Livne, 1973.

Freshly drawn blood (30–40 ml) from a normal volunteer was centrifuged and the erythrocytes were removed and washed three times with isotonic NaCl. After addition of an equal volume of distilled water to the separated cells the haemolysate so obtained was centrifuged at 27,000 *g* for 20 min. The cell free homogenate was then divided into several aliquots and to each was added an equal volume of a buffer solution such that the final concentrations of the incubation mixture were as follows: glucose = 2.4 mmol/l; ATP = 0.6 mmol/l; ADP = 0.6 mmol/l; NAD = 0.15 mmol/l; NADH = 0.15 mmol/l; NADP = 0.6 mmol/l; $MgCl_2$ = 4 mmol/l; phosphate buffer at pH 7.8 = 3 mmol/l; 3-phosphoglyceric acid = 0.2 mmol/l; KCl = 150 mmol/l. To some of these aliquots were added various concentrations of LiCl.

The incubation mixture was equilibrated for 5 min at 37°C and timed samples (0.1 ml) were then taken. Glucose was determined by a modification of the glucose oxidase-PERID method (Werner, Rey & Wielinger, 1970): haemoglobin by the method of Van Kampden & Zijlstra (1961).

Lithium consistently inhibited the rate of glucose disappearance though interindividual differences were seen in five subjects. In all cases 5 mmol/l lithium caused inhibition. 30 mmol/l lithium was used to ensure more consistent results in the two subjects who were studied more closely (Table 1).

We conclude that lithium does inhibit glycolysis in erythrocytes.

References

BASHAN, N., MOSES, S., LIVNE, A. (1973). The effect of Na^+ and K^+ on glycolysis in reconstituted hemolysates. *FEBS Letts.*, **36**, 213–216.

BIRCH, N.J. (1978) Lithium in Medicine. In *New Trends in Bio-inorganic Chemistry*, Ed. Williams, R.J.P., da Silva, J.J.R.F., pp. 389–435, London, Academic Press.

KAJDA, P.K., BIRCH, N.J., O'BRIEN, M.J., HULLIN, R.P. (1979). Rat brain pyruvate kinase purification and effects of lithium. *J. Inorgan. Biochem.*, **11**, 361–366.

VAN KAMPDEN, E.J., ZIJLSTRA, W.G. (1961) Standardisation of haemoglobinometry. II The haemoglobincyanide method. *Clin. Chim. Acta*, **6**, 538–544.

WERNER, W., REY, H.-G. WIELINGER, H. (1970). Über die Eigenschaften eines neuen Chromogens für die Blutzuckerbestimmung nach der GOD/POD Methode. *Z. analyt. chem.*, **252**, 224–228.

Table 1 Inhibition by 30 mmol/l LiCl of glucose utilization (expressed as μmol glucose/gm Hb/h) compared with lithium = 0 mmol/l in haemolysates from two normal subjects (mean ± s.d.)

Subject	*No. of expts.*	*% inhibition* 0–1 h	1–2 h	0–2 h
A	6	51.8 ± 17.3	40.5 ± 7.7	46.9 ± 8.7
B	3	5.5 ± 3.5	27.8 ± 10.3	23.3 ± 15.7

The cardiovascular effects of intracisternal Substance P in the normotensive rabbit

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Dense networks of Substance P immunoreactive nerve terminals are found in the nucleus of the solitary tract in the brain stem (Hökfelt, Johansson, Kellerth, Ljungdahl, Nilsson, Nygards & Pernow, 1976). Gillis, Helke, Hamilton, Norman & Jacobowitz (1980) have suggested that Substance P may be a neurotransmitter in primary baro- and chemoreceptor afferents at the level of this nucleus. In this study the interaction of intracisternally (i.c.) administered Substance P with other neurotransmitter systems, which may be involved in the central connections of the baroreceptor reflex has been investigated.

Mean arterial pressure (MAP) and heart rate (HR) were measured directly in pentobarbitone (30 mg/kg) anaesthetized rabbits ($n = 8$). The i.c. administration of Substance P (0.2–10 ng/kg) caused a dose-dependent increase in MAP and bradycardia in contrast to the depressor effects observed after intravenous (i.v.) administration of higher doses. The maximum rise occurred 2 min after i.c. injection and gradually returned to control values by 15 minutes. Both responses were attenuated by the prior injection of i.v. pentolinium (3 mg/kg).

To investigate if these changes caused by Substance P involve central catecholaminergic systems, the effect of the peptide (5 ng/kg) on MAP and HR were measured 7 days after 6-hydroxydopamine (500 μg/kg i.c.) and 15 min after prazosin (20 μg/kg i.c.) and yohimbine (1 mg/kg i.v.). The latter are α adrenoceptor antagonists, with differing affinities for α_1 and α_2 receptors. 6-Hydroxydopamine pretreatment had no effect on the blood pressure rise caused by Substance P, but significantly attenuated ($P < 0.01$) the bradycardia. Prazosin also had no effect on the increase in pressure but blocked the bradycardia ($P < 0.01$). Yohimbine, the α_2 receptor antagonist, had no effect on the maximum pressor response caused by Substance P, but increased its duration (rise at 15 min being 10.1 ± 5.7 mmHg (mean ± s.d.) in the presence of yohimbine and 1.6 ± 7 mmHg after peptide alone ($P < 0.05$)). Yohimbine also attenuated the bardycardia ($P < 0.01$).

As enkephalin mechanisms may also be involved in the central regulation of baroreceptor reflexes (Petty & Reid, 1980) the responses to Substance P have been investigated after pretreatment with naloxone, an opiate antagonist. Naloxone (200 μg/kg i.v.) administered 10 min prior to the peptide (5 ng/kg i.c.) prevented the decrease in HR ($P < 0.01$), but potentiated the rise in pressure, which was 26.5 ± 7.6 mmHg in the presence of naloxone, compared to 18.3 ± 9.7 mmHg after Substance P alone ($P < 0.05$), 2 min after peptide administration. Blood pressure returned to control values by 15 minutes.

The cardiovascular effects of i.c. injection of Substance P are mediated by peripheral autonomic mechanisms. The central effects of Substance P on heart rate are prevented by opiate antagonists and by destruction of central catecholamine containing neurones. However, the central pressor action is uninfluenced or enhanced by these manoeuvres. These studies and preliminary observations indicating that baroreflex sensitivity is reduced by central Substance P confirm that this putative peptide transmitter may exert important modulatory effects on cardiovascular regulation in the brain stem.

References

GILLIS, R.A., HELKE, C.J., HAMILTON, B.L., NORMAN, W.P. & JACOBOWITZ, D.M. (1980). Evidence that Substance P is a neurotransmitter of baro- and chemoreceptor afferents in nucleus tractus solitarius. *Brain Res.*, **181**, 476–481.

HÖKFELT, T., JOHANSSON, O., KELLERTH, J.O., LJUNGDAHL, Å., NILSSON, G., NYGÅRDS, A. & PERNOW, B. (1976). In *Substance P*, ed. von Euler, U.S. & Pernow, B., pp. 117–145, New York, Raven Press.

PETTY, M.A. & REID, J.L. (1980). The effects of opiates on baroreceptor reflex sensitivity in the normotensive rabbit. Paper presented to *Br. J. Pharmac. Soc., Bristol*, 1980.

Creatinol O-phosphate is a potential intracellular buffer

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Creatinol O-phosphate pretreatment in rats reduces the cardionecrotic action of isoprenaline (Godfraind & Sturbois, 1979) and prevents the extension of the size of the infarct evoked by left coronary artery ligation (Doncquier & Godfraind, 1980). Clinical trials have shown that it reduces the CPK serum levels in acute myocardial infarction (Knippel *et al.*, 1979). Furthermore, it is effective against cardiac arryhthmia associated with ischaemic heart disease (Cadel *et al.*, 1979). Experiments on anesthetized dogs and on rat isolated atria have shown that creatinol O-phosphate maintained heart contractility when isoprenaline was in excess (Godfraind *et al.*, 1979). Other reports show that it improved lactate utilization in dogs and maintained the contractility of the isolated heart in hypoxia (Marchetti & Merlo, 1978).

Creatinol O-phosphate appears therefore to be efficient in hypoxic and ischaemic conditions. Although hypoxia and anoxia are different situations (Williamson *et al.*, 1976), they are characterized by a metabolic predominance of the anaerobic glycolysis associated with cell acidification. The lowering of intracellular pH interferes with the effectiveness of the glycolysis and could be responsible for tissue alteration (Williamson *et al.*, 1976).

We have measured changes in pH following the addition of HCl in a solution containing Mg creatinol O-phosphate and have compared the titration curves with those obtained with sodium bicarbonate and potassium phosphate. Creatinol O-phosphate appeared to buffer between pH 6.8 and pH 5.4 in a region slightly more acidic than bicarbonate and phosphate. In this buffer zone, a 0.1 pH change required 88 mEq HCl/mol creatinol O-phosphate as compared to 65 for bicarbonate and 55 for phosphate.

Since it has been shown that creatinol O-phosphate enters the cell and since its buffering capacity is in the range of intracellular pH, the prevention of intracellular acidosis could contribute to the anti ischaemic action of this drug.

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References

CADEL, A., PALUMBO, A., ZERILLI, G., PRIA, R., FANCIULLI, R., CONVERSANO, S. & GALBIATI, R. (1979). Antiarrhythmic effectiveness of creatinol O-phosphate in man. *Arzneim. Forsch.*, **29**, 1485–1487.

DONCQUIER, J. & GODFRAIND, T. (1980). Reduction by creatinol O-phosphate of heart CPK loss after coronary artery ligation in rats. Submitted.

GODFRAIND, T., GHYSEL-BURTON, J. and MERLO, L. (1979). The action of creatinol O-phosphate on the inotropic effect of isoprenaline in isolated rat atria and in anaesthetized dogs. *Arzneim. Forsch.*, **29**, 1465–1467.

GODFRAIND, T. & STURBOIS, X. (1979). An analysis of the reduction by creatinol O-phosphate of the myocardial lesions evoked by isoprenaline in the rat. *Arzneim. Forsch.*, **29**, 1457–1464.

KNIPPEL, M., BANA, G. & PUSTERLA, G.L. (1979). Effect of creatinol O-phosphate on serum enzymes in acute myocardial infarction. *Arzneim. Forsch.*, **29**, 1480–1482.

MARCHETTI, G. & MERLO, L. (1978). Effects of creatinol O-phosphate (COP) on haemodynamics and cardiac metabolism in conscious and anaesthetized dogs. *Arzneim. Forsch.*, **28**, 1708–1711.

WILLIAMSON, J.R., SCHAFFER, S.W., FORD, CH. & SAFER, B. (1976). Contribution of tissue acidosis to ischemic injury in the perfused rat heart. *Circulation*, **53**, Suppl. I, 3–14.

Reduction by creatinol O-phosphate of heart CPK loss after coronary artery ligation in rats

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Creatinol O-phosphate has been shown to protect rats against the cardionecrotic action of isoprenaline (Godfraind & Sturbois, 1979). During clinical trials, it has been reported that the increase in serum CPK level observed after a heart infarction was reduced in patients treated by creatinol O-phosphate (Knippel *et al.*, 1979). We have therefore examined the influence of pretreatment by this drug on the CPK loss induced in rat heart by coronary artery ligation.

Wistar rats weighing 360 to 400 g were pretreated or not during 4 days by Mg creatinol O-phosphate (10 mg/kg) injected i.p. The left coronary artery was

ligated according to the method described by Selye *et al.* (1960). The animals were killed 6 h later. The hearts were homogenized. The CPK activity was assayed with a commercial kit (Boehringer) in the supernatant of a 10 min centrifugation at 2,500 *g*. The results were expressed in CPK units per mg protein.

The ligation of the left coronary artery resulted in a 41% decrease of the heart CPK content: from 21.1 ± 0.66 U/mg ($n = 10$) in Sham operated rats to 12.4 ± 0.68 U/mg ($n = 10$) in coronary ligated ($P < 0.01$). In the group pretreated with creatinol O-phosphate, the CPK activity decreased by only 30% since the homogenate contained 14.8 U/mg ($n = 10$); the difference with untreated rats was significant ($0.001 < P < 0.01$).

The CPK loss may be considered as an index of the heart infarct size. The present results indicate that pretreatment with creatinol O-phosphate prevents the extension of the infarct following left coronary artery ligation in rats. In view of the clinical report that it also reduced the serum CPK levels in acute myocardial infarction, it is likely that this action also occurs in man.

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References

GODFRAIND, T. & STURBOIS X. (1979). An analysis of the reduction by creatinol O-phosphate of the myocardial lesions evoked by isoprenaline in the rat. *Arzneim. Forsch.*, **29**, 1457–1464.

KNIPPEL, M., BANA, G. & PUSTERLA, G.L. (1979). Effect of creatinol O-phosphate on serum enzymes in acute myocardial infarction. *Arzneim. Forsch.*, **29**, 1480–1482.

SELYE, H., BAJUSZ, E., GRANO, S. & MENDELL, P. (1980). Simple techniques for the surgical occlusion of coronary vessels in the rat. *Angiology*, **11**, 398–407.

Exploratory, social and d-amphetamine induced behaviour in spontaneously hypertensive rats

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The development of hypertension in the spontaneously hypertensive rat (SHR) is thought to be associated with central hyperactivity to emotional and environmental stimuli (Hallbäck & Weiss, 1977). There is however little information available concerning the behavioural characteristics of SHR's compared with strains of normotensive rat. The present communication is part of a study of exploratory and social behaviour and monoaminergic neuronal function in SHR (Wistar-Kyoto), Wistar and Sprague–Dawley male albino rats.

Rats (24–36 of each strain) were isolated on day 1 and tested for their exploratory and social behaviour on day 6. On day 7 individual rats were given either saline, (+)-amphetamine (2.5 mg/kg) or tranylcypromine (10 mg/kg) plus L-tryptophan (40 mg/kg) 30 min later. Exploratory and social behaviour were monitored together using a pair of rats of the same strain, within a 10 gram weight range, that had not met previously. They were placed for 10 min in a box (55 × 65 × 64 cm), the floor of which was a head-dip board, under light 'unfamiliar' (the box was strange to each animal) and dark 'familiar' conditions (each rat had separately explored the box on two previous occasions (File & Hyde, 1978) and a video-recording made of the events. The events were analyzed into exploratory behaviour (head dips), active social contact and aggressive behaviour. The effects of tranylcypromine plus L-tryptophan and d-amphetamine were monitored using a behavioural score (Marsden & Curzon, 1978) and a doppler shift radar activity monitor. Blood pressure (tail cuff) was measured on the day the rats were isolated and 6 days after isolation.

Blood pressure (BP) of SHR's (184 mmHg ± 5, $n = 24$) were significantly higher than Wistar (133 ± 7, $n = 24$) or Sprague–Dawley (132 ± 4, $n = 24$) rats before isolation. Isolation significantly increased the BP of Wistar (161 ± 8, $n = 12$) and Sprague–Dawley rats (148 ± 5, $n = 12$) as previously reported (Gardiner & Bennett, 1977) and also further increased the BP of SHR's (208 ± 5, $n = 12$). There were no significant differences between Wistar and Sprague–Dawley rats in terms of active social, exploratory or aggressive behaviour. SHR's however showed significantly more exploratory activity under light unfamiliar conditions compared to either Wistar ($P < 0.001$) or Sprague–Dawley ($P < 0.05$) and dark familiar compared to Sprague–Dawley ($P < 0.05$). The SHR's were also more aggressive, under both experimental conditions, than either Wistar or Sprague–Dawley rats ($P < 0.05$). While there was no significant differences in active social contact between any of the

strains under dark familiar conditions the SHR's showed significantly less social contact than either Wistar ($P < 0.001$) or Sprague–Dawley ($P < 0.01$) rats under the light unfamiliar (high stress) conditions. There was no significant difference in the behavioural response produced by L-tryptophan plus tranylcypromine between the three strains but d-amphetamine (2.5 mg/kg) produced a significantly smaller increase in activity in the SHR's ($P < 0.05$).

A previous study has shown hyper-exploratory activity in pre-hypertensive SHR's (Knardahl & Sagvolden, 1979). The present results indicate that this trend, together with hyper-reactivity to a 'high stress' situation, continues after the development of hypertension and that it may be associated with pre- or post-synaptic changes of central catecholamine neurones.

References

FILE, S.E. & HYDE, J.R.G. (1978). Can social interaction be used to measure anxiety? *Br. J. Pharmac.*, **62**, 19–24.

GARDINER, S.M. & BENNETT, T. (1977). The effects of short-term isolation on systolic blood pressure and heart rate in rats. *Med. Biol.*, **55**, 325–329.

HALLBÄCK, M. & WEISS, L. (1977). Mechanisms of spontaneous hypertension in rats. *Med. Clin. North Am.*, **61**, 593–609.

KNARDAHL, S. & SAGVOLDEN, T. (1979). Open field behaviour of spontaneously hypertensive rats. *Behav. & Neural Biol.*, **27**, 187–200.

MARSDEN, C.A. & CURZON, G. (1978). The contribution of tryptamine to the behavioural effects of L-tryptophan in tranylcypromine treated rats. *Psychopharmac.*, **57**, 71–76.

Comparative studies on the cardiovascular and behavioural actions of guanabenz and clonidine

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Guanabenz is a highly selective α_2-adrenoceptor agonist (Doxey & Hersom, 1980) which like clonidine is believed to lower blood pressure via actions on central α-adrenoceptors (Baum & Shropshire, 1976). Guanabenz, in common with clonidine causes sedation in man (Tester-Dalderup, 1977). In this study, therefore, the cardiovascular and behavioural effects of guanabenz and clonidine have been compared in female normotensive Sprague Dawley rats (> 180 g).

Cardiovascular studies were performed in pentobarbitone anaesthetized rats as described previously (Doxey & Hersom, 1980). Each rat received only one dose of clonidine (1–30 μg/kg, i.v.) or guanabenz (1–100 μg/kg, i.v.), and in antagonist experiments, piperoxan (10 mg/kg, i.v.), phentolamine (1 mg/kg, i.v.) or phenoxybenzamine (3 mg/kg, i.v.) were administered 15 min before the agonists clonidine or guanabenz.

Intravenous administration of guanabenz or clonidine ($n = 5$) elicited a transient pressor response followed by a sustained hypotension and bradycardia. The doses of clonidine and guanabenz required to reduce resting blood pressure by 25% were 3.6 and 6.1 μg/kg, i.v. respectively, calculated from the results by linear regression analysis.

Administration of piperoxan, phentolamine or phenoxybenzamine all significantly ($P < 0.05$) reduced resting blood pressure. Intravenous pretreatment with the α-adrenoceptor antagonist piperoxan significantly attenuated ($P < 0.01$) hypotensive and bradycardic actions of clonidine (30 μg/kg, i.v.) and guanabenz (100 μg/kg, i.v.) whereas pretreatment with either phentolamine or phenoxybenzamine did not significantly ($P > 0.05$) alter the hypotensive and bradycardic actions of guanabenz and clonidine.

Behavioural status was assessed by counting grid crossings in the open field test ($n = 10$), and measuring the time that trained rats ($n = 6$–8) could remain on a horizontal drum, 30.5 cm in diameter, rotated at 4.7 revolutions/min. Rats pretreated 30 min previously with guanabenz (0.3–3 mg/kg, i.p.) or clonidine (0.03–0.3 mg/kg, i.p.) showed a dose-dependent decreased locomotor activity in the open field test, and decreased time on the rotating drum. The doses of the agonists required to reduce locomotor activity by 50%, and the time animals remained on the drum by 50% were 0.23 and 0.10 mg/kg, i.p. for clonidine and 0.92 and 0.42 mg/kg, i.p. for guanabenz respectively.

The potency of guanabenz relative to clonidine was similar in both the cardiovascular and behavioural tests used in this study. These results suggest that guanabenz, like clonidine (Hersom, Finch & Metcalf, 1978) may reduce blood pressure and heart rate by an action on central α-adrenoceptors similar in nature to peripheral α_2-adrenoceptors. The sedation observed with both guanabenz and clonidine may also be related to agonist activity at central α-adrenoceptors

which are considered to have a similar pharmacological profile to peripheral α_2-adrenoceptors (Drew, Gower & Marriott, 1977; Birch, Clough, Hatton & Wheatley, 1979).

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References

BAUM, T. & SHROPSHIRE, A.T. (1976). Studies on the centrally mediated hypotensive activity of guanabenz. *Eur. J. Pharmac.*, **37**, 31–44.

BIRCH, P., CLOUGH, D.P., HATTON, R. & WHEATLEY, D.J. (1979). Hypotensive and sedative properties of α-adrenoceptor agonists: relation to pre- and postsynaptic stimulation. *Br. J. Pharmac.*, **68**, 107P–108P.

DOXEY, J.C. & HERSOM, A.S. (1980). The relationship between the selectivity of agonists for presynaptic α_2-adrenoceptors and their cardiovascular responses in the anaesthetised rat. *Br. J. Pharmac.* (in press).

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.

HERSOM, A.S., FINCH, L. & METCALF, G. (1978). Interactions between clonidine and α-adrenoceptor antagonists. *Proc. 7th Int. Congr. Pharmac., (Paris)*, **Abstract** 2713.

TESTER-DALDERUP, C.B.M. (1977). Hypotensive drugs. In: *Side-effects of drugs Annual 1*, 1977, Ed. M.N.G. Dukes pp. 164–179 Excerpta Medica, Amsterdam-Oxford.

Spiperone contracts the human basilar arterial strip *in vitro*: possible interaction with 5-hydroxytryptamine receptors?

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Recent evidence suggests that the receptor sites for 5-hydroxytryptamine (5-HT) on the human basilar artery are different from other vascular beds (Forster & Whalley, 1980). It has been demonstrated that the human basilar arterial strip is much more sensitive to 5-HT than is the rat aortic strip (Forster, Whalley, Mohan & Dutton, 1980) and that antagonists of 5-HT such as methysergide, cyproheptadine and methergoline do not antagonize 5-HT on the human basilar artery *in vitro* in a competitive fashion (Forster & Whalley, 1980). This receptor may be similar to that described by Apperley, Feniuk, Humphrey & Levy (1980) on the dog saphenous vein.

This study further evaluates and compares the 5-HT receptor on the human basilar arterial and rat aortic strip *in vitro*. The effect of spiperone was investigated since this drug has been shown to bind specifically to a distinct population of 5-HT receptors in rat brain (Peroutka & Snyder, 1979) and to possess 5-HT-antagonistic actions on various behavioural syndromes such as the 5-hydroxytryptophan induced wet dog shakes in rats (Maj, Baran, Bigajska, Rogóz & Skuza, 1978).

In addition, the effects of desensitization induced by repeated administration of 5-HT or spiperone were compared on the human basilar artery and rat aortic strip *in vitro*.

Rat aortic and human basilar arterial strips were prepared as described previously (Forster *et al.*, 1980). Spiperone was found to be a partial agonist on the human basilar artery producing a maximum response of 70% of the 5-HT-maximum. Spiperone was ineffective on the rat aorta.

Rapid desensitization of the human basilar artery and rat aorta was achieved by repeated (every 10 min) administration of 5-HT (10^{-4}M—a concentration selected following preliminary experiments utilising several concentrations of 5-HT). 5-HT was applied over a period of up to 4 hours. On the rat aorta the responses to 5-HT were almost completely abolished over the time period studied. In contrast on the human basilar artery, desensitization was never complete and in addition was partially reversible. Responses to a standard concentration of noradrenaline were unaffected during the desensitization period. Responses of the human basilar artery to a standard concentration of spiperone (2.5×10^{-6} M) applied after maximum desensitization and after partial recovery from desensitization of 5-HT were abolished. After allowing the tissues to recover for 1 h it was found that responses to 5-HT and spiperone were almost equivalent to those seen before desensitization.

These results suggest that spiperone may be acting on 5-HT-receptors on the human basilar artery. In addition the phenomenon of desensitization to 5-HT on the human basilar artery appears to be different from that on the rat aorta.

References

APPERLEY, E., FENIUK, W., HUMPHREY, P.P.A. & LEVY, G.P. (1980). Evidence for two different types of receptor for 5-hydroxytryptamine in dog isolated vasculature. *Br. J. Pharmac.*, **68**, 215–224.

FORSTER, C. &. WHALLEY, E.T. (1980). Comparison of the

5-hydroxytryptamine-induced contraction of human basilar arterial and rat aortic strips *in vitro*. *Br. J. Pharmac.*, In Press.

FORSTER, C., WHALLEY, E.T., MOHAN, J. & DUTTON, F. (1980). Vascular smooth muscle response to fibrinogen degradation products and 5-hydroxytryptamine: possible role in cerebral vasospasm in man. *Br. J. Clin. Pharmac.*, In Press.

MAJ, J., BARAN, L., BIGAJSKA, K., ROGÓZ, Z. & SKUZA, G. (1978). The influence of neuroleptics on the behavioural effect of 5-hydroxytryptophan. *Pol. J. Pharmacol. Pharm.*, **30**, 431–440.

PEROUTKA, S.J. & SNYDER, S.H. (1979). Multiple serotonin receptors: differential binding of [^{3}H]-5-hydroxytryptamine, [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol. *Mol. Pharmacol.*, **16**, 687–699.

Differences in the effects of the calcium antagonists nimodipine (BAY e 9736) and bencyclan on cerebral and peripheral vascular smooth muscle

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At least two separate 'channels' appear to be involved in the calcium influx necessary for sustained contraction of vascular smooth muscle: (1) potential sensitive channels (PSCs) operated by depolarization, e.g. by KCl, and (2) receptor operated channels (ROCs) operated by vasoactive agonists (Bolton, 1979; Meisheri *et al.*, 1980). Calcium antagonists such as verapamil and nifedipine (Schümann *et al.*, 1975) or nimodipine (Towart & Kazda, 1979) potently inhibit K^+-induced contractions of rabbit isolated aortic strip (presumably due to block of calcium influx through PSCs), but have no effect on noradrenaline-induced contractions in this tissue. The peripheral vasodilator bencyclan behaves similarly (Table 1a) and is presumably also a calcium antagonist.

We have previously shown that serotonin-induced contractions of the rabbit saphenous artery are similarly unaffected by nimodipine, but that in contrast serotonin contractions of the rabbit basilar artery are powerfully inhibited by nimodipine (Towart & Kazda, 1980; see also Table 1b). We have now examined the effect of bencyclan on these vessels.

Basilar and saphenous arteries were obtained from Chinchilla rabbits (2.5–3.5 kg) sacrificed by pentobarbitone overdose. Ring segments (3–4 mm) were suspended isometrically between two stainless steel wires in oxygenated (95% O_2 + 5% CO_2) Krebs Henseleit solution at 37°C, and tension measured with a strain gauge. After equilibration (2 h) reproducible contractions were induced by addition of serotonin (2.5×10^{-7} M) for 20 minutes.

The effects of nimodipine (2.4×10^{-8} M) and of bencyclan (2.5×10^{-6} M) are shown in Table 1(b). Nimodipine inhibited the sustained contraction of the

Table 1(a) 50% inhibitory doses (ID_{50}) of nimodipine and bencyclan on contractions of the rabbit isolated aortic strip induced by noradrenaline or by KCl

	ID_{50} (*noradrenaline*)	ID_{50} *KCl*)
Nimodipine	$\gg 2.4 \times 10^{-5}$ M	2.9×10^{-9} M
Bencyclan	$\gg 2.5 \times 10^{-5}$ M	3×10^{-6} M

Table 1(b) Inhibition of sustained serotonin-induced contractions of rabbit isolated arterial rings, expressed as % of previous control contractions in presence of solvent

	Saphenous artery	*Basilar artery*
Nimodipine (2.4×10^{-8} M)	29% ± 6.7** ($n = 4$)	65.3% ± 4.9 ($n = 4$)
Bencyclan (2.5×10^{-6} M)	86.5% ± 3.3 ($n = 4$)	23.8% ± 8.4* ($n = 4$)

Values expressed as mean ± s.e. mean. Significance of difference between the inhibitions produced by the antagonists: * $P < 0.05$; ** $P < 0.001$.
(Student's *t*-test, two-tailed.)

basilar artery by 65%, but had little effect on the saphenous artery. Bencyclan in contrast inhibited mainly the saphenous artery. As the serotonin-induced contractions of one of the vessels is little affected by each drug, it is unlikely that either drug is acting at the receptor level. It is suggested that the serotonin receptor-operated calcium influx channels in the saphenous and basilar arteries are different. The former appear to be more readily blocked by bencyclan, and the latter are more readily blocked by nimodipine.

References

BOLTON, T.B. (1979). Mechanisms of action of transmitter and other substances on smooth muscle. *Physiol. Rev.*, **59**, 606–718.

MEISHERI, K.D., PALMER, R.F. & VAN BREEMEN, C. (1980). The effects of amrinone on contractility, Ca^{2+} uptake and cAMP in smooth muscle. *Eur. J. Pharmac.*, **61**, 159–165.

SCHÜMANN, H.J., GÖRLITZ, B.D. & WAGNER, J. (1975). Influence of papaverine, D600 and nifedipine on the effects of noradrenaline and calcium on the isolated aorta and mesenteric artery of the rabbit. *Arch. Pharmacol.*, **289**, 409–418.

TOWART, R. & KAZDA, S. (1979). The cellular mechanism of action of nimodipine (BAY e 9736), a new calcium antagonist. *Br. J. Pharmac.*, **67**, 409P–410P.

TOWART, R. & KAZDA, S. (1980). Selective inhibition of serotonin-induced contractions of rabbit basilar artery by nimodipine (BAY e 9736). *IRCS Med. Sci.*, **8**, 206.

A comparison of flunarizine and cinnarizine

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Flunarizine, a difluoroderivative of cinnarizine shares with the parent compound the property to inhibit the contraction evoked by calcium in depolarized smooth muscle (Van Nueten *et al.*, 1978; Godfraind, 1979). Since it was proposed that the pharmacological profile of the two compounds could be different (Van Nueten *et al.*, 1978), we have compared their antagonism to the action of noradrenaline in rat aorta and mesenteric arteries.

Rings of aorta were prepared as reported elsewhere (Godfraind, 1979). The mesenteric bed was perfused according to McGregor (1965) and its resistance was increased either by the injection of various amounts of noradrenaline or by the continuous perfusion of a fixed concentration of this catecholamine. The physiological solution was composed as follows: (mM) NaCl 112, KCl 5, $NaHCO_3$ 25, KH_2PO_4 1, $MgSO_4$ 1.2, $CaCl_2$ 1.25, glucose 11.5. It was gassed at 37°C by a mixture of 95% O_2 and 5% CO_2 and contained or not various concentrations of cinnarizine or flunarizine.

The two drugs appeared to be equipotent on the aorta. The maximum reduction of the noradrenaline contraction was reached with 3×10^{-6} M which left unaltered 50% of the response. For the two drugs, IC_{50} was equal to 10^{-7} M.

In the mesenteric bed, the two drugs depressed the increase in tension evoked by a noradrenaline perfusion. A pretreatment of 90 min with 10^{-6} M of the two drugs nearly abolished the maximum response. IC_{50} was equal to 2×10^{-7} M for cinnarizine and to 4×10^{-8} M for flunarizine. Bolus injections of noradrenaline were blocked by flunarizine but only slightly affected by cinnarizine.

The increase in perfusion pressure evoked by a noradrenaline perfusion depends on the concentration of calcium in the physiological solution whereas the transient increase in pressure evoked by the injection of a noradrenaline bolus is maintained for long periods in Ca-deprived solution. It is therefore likely that two different mechanisms are activated in mesenteric arteries according to the mode of administration of noradrenaline. These two mechanisms are sensitive to flunarizine but not to cinnarizine which only blocks the tonic contraction dependent on the calcium concentration of the perfusion fluid.

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References

GODFRAIND, T. (1979). Alternative mechanisms for the potentiation of the relaxation evoked by isoprenaline in aorta from young and aged rats. *Eur. J. Pharmacol.*, **253**, 273–279.

MCGREGOR, D.D. (1965). The effect of sympathetic nerve stimulation of vasoconstrictor responses in perfused mesenteric blood vessels of the rat. *J. Physiol.*, (*Lond.*) **177**, 21–30.

VAN NUETEN, J.M., VAN BEEK, J. & JANSSEN, P.A.J. (1978). The vascular effects of flunarizine as compared with those of other clinically and vasoactive substances. *Arzneim. Forsch.*, **28**, 2082–2087.

Tolerance to the inhibition by morphine of bradycardia induced by vagal stimulation in the rat

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Morphine dependence studies in the rat have generally utilized behavioural signs (Buckett, 1964) or biochemical indices (Takemori, 1974) of morphine withdrawal in order to assess the degree of physical dependence acquired. Surprisingly little attention has been paid to the effects of morphine on the cardiovascular system with respect to tolerance and dependence. This preliminary report presents evidence to show that the acute inhibitory effect of morphine on the bradycardia induced by vagal stimulation (Kosterlitz & Taylor, 1959) is diminished after chronic morphine pretreatment in the rat.

Rats of either sex and body weight 240–310 g were injected i.p. either with morphine HCl twice daily as follows: day 1, 30 mg/kg; day 2, 100 mg/kg; and day 3, 300 mg/kg (morphine-treated; $n = 10$) or with 0.9% saline (control; $n = 10$). At 16–20 h after the last injection the rats were anaesthetized with chloralose (100 mg/kg i.p.) and sodium pentobarbitone (30 mg/kg i.p.) and were artificially ventilated. Heart rate was recorded from the ECG and the blood pressure was monitored from a cannulated carotid artery. The exposed and severed cervical vagus nerve was bathed in liquid paraffin and stimulated via a bipolar platinum electrode with rectangular pulses of 0.5 ms duration and supramaximal voltage (usually 8 V), at a frequency of 2, 10, 4 and 20 Hz (in that order) for 10 s of every 2 minutes. Intravenous saline or doses of morphine were administered i.v. in a randomized order via an external jugular vein at intervals of not less than 20 minutes.

The resting heart rate of morphine-treated rats (279 ± 10 beats/min) was significantly higher ($P < 0.05$) than that of controls (241 ± 11 beats/min). Morphine HCl (0.5 or 2.0 mg/kg i.v.) depressed the resting heart rate in control rats to a significantly greater degree ($P < 0.001$) than in morphine-treated rats indicating tolerance to this effect of the drug. The reduction in heart rate in response to vagal stimulation was frequency-dependent from 2 to 20 Hz in both morphine-treated and control rats. In the morphine-treated animals the reduction in number of beats/min was significantly greater ($P < 0.05$) at frequencies of 10 and 20 Hz than in controls. However as the resting heart rate of these animals was higher, the heart rate induced by vagal stimulation did not differ significantly between control and morphine-treated rats. Morphine HCl (0.5 mg/kg, i.v.) significantly antagonized ($P < 0.01$) the reduction in heart rate induced by 20 Hz stimulation in control but not in morphine-treated rats, although increasing the dose to 2 mg/kg elicited a significant ($P < 0.01$) antagonism in morphine-treated animals. At the lower rates of stimulation, morphine HCl (1.0 and 2.0 mg/kg i.v.) was significantly ($P < 0.05$) effective in antagonizing vagal bradycardia in control but not in morphine-treated rats.

It is concluded that the inhibitory action of morphine on vagally-induced bradycardia in the rat is one which is susceptible to tolerance. The correlation of it with other aspects of dependence and tolerance which are acquired at different rates (Fernandes *et al.*, 1977) is under investigation.

References

BUCKETT, W.R. (1964). A new test for morphine-like physical dependence (addiction liability) in rats. *Psychopharmacologia*, **6**, 410–416.

FERNANDES, M., KLUWE, S. & COPER, H. (1977). The development of tolerance to morphine in the rat. *Psychopharmacology*, **54**, 197–201.

KOSTERLITZ, H.W. & TAYLOR, D.W. (1959). The effect of morphine on vagal inhibition of the heart. *Br. J. Pharmac.*, **14**, 209–214.

TAKEMORI, A.E. (1974). Biochemistry of drug dependence. *Ann. Rev. Biochem.*, **43**, 15–33.

Demonstration of simultaneous α_1-, α_2-, β_1- and β_2-adrenoceptor mediated effects of phenylephrine in the cardiovascular system of the pithed rat

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Sub-classification of adrenoceptors relies on the use of 'selective' agonists and antagonists. The recent demonstration of α_2-adrenoceptors mediating pressor responses in the pithed rat relied, in part on the 'relative' resistance of phenylephrine (an 'α_1-agonist') to yohimbine (an 'α_2-antagonist'): this served as a control compared with the susceptibility to yohimbine of xylazine and guanabenz ('α_2-agonists') (Docherty & McGrath, 1980). However, to establish the physiological role of such receptors or to make any quantitative assessment of the α_1- or α_2-adrenoceptor antagonism of test drugs it is necessary to establish more precisely the degree of 'selectivity' of phenylephrine. It is already known that phenylephrine can act as a β-adrenoceptor agonist and an indirect sympathomimetic (Santon, Dungan & Lish, 1965; Luchelli-Fortis & Langer, 1974). Furthermore, the α_2-adrenoceptor agonism of adrenaline on vascular smooth muscle became clearer when its β-adrenoceptor agonism was eliminated (Flavahan & McGrath, 1980a, b). The cardiovascular effects of phenylephrine were, thus, compared with those of amidephrine, which has been shown, in other preparations, to lack β- or α_2-agonism (Santon *et al.*, 1965; Butler & Jenkinson, 1978).

Male Wistar rats were pithed by the method of Gillespie, McLaren & Pollock (1970) and respired with O_2. Carotid arterial blood pressure and heart rate were monitored and drugs injected via a jugular vein. A maximum of one dose of amidephrine or two of phenylephrine was administered to each rat due to the long lasting effects of these drugs.

In the absence of other drugs, phenylephrine produced a dose-dependent (diastolic) pressor response. Following prazosin (1 mg/kg) phenylephrine produced depressor responses, i.e. 'phenylephrine reversal' occurred. Subsequent addition of propranolol (1 mg/kg) abolished this depressor response and allowed a pressor response to reappear. Propranolol alone did not significantly affect the dose/response curve to phenylephrine indicating that the β-effect is normally dominated by the α-effect. The pressor response to phenylephrine which remained after prazosin and propranolol was antagonized by rauwolscine (1 mg/kg). This indicates an α_2-adrenoceptor-mediated pressor effect of phenylephrine since rauwolscine is selective for α_2-adrenoceptors (Weitzell, Tanaka & Starke, 1979). Rauwolscine alone produced a small but significant antagonism of the pressor effect of phenylephrine. Phenylephrine produced a dose-dependent increase in heart rate over the same dose range as the pressor effects; this was antagonized by propranolol.

Amidephrine produced a dose-dependent pressor response but little increase in heart rate. The pressor response was antagonized by prazosin (1 mg/kg) but was unaffected by rauwolscine (1 mg/kg) or propranolol (1 mg/kg). No depressor response indicative of 'amidephrine reversal' was found after prazosin.

It is concluded that the vascular effect of phenylephrine is predominantly pressor α_1- but that it also stimulates pressor α_2-adrenoceptors and depressor β_2-adrenoceptors whose effects will complicate quantitative analysis of the effects of 'mixed' antagonists.

References

BUTLER, M. & JENKINSON, D.H. (1978). Blockade by WB4101 of α-adrenoceptors in the rat vas deferens and guinea-pig *taenia caeci*. *Eur. J. Pharmac.*, **52**, 303–311.

DOCHERTY, J.R. & McGRATH, J.C. (1980). A comparison of pre- and post-junctional potencies of several alpha-adrenoceptor agonists in the cardiovascular system and anococcygeus of the rat: evidence for 2 types of post junctional alpha-adrenoceptor. *Naunyn-Schmiedeberg's Arch. Pharmacol.* (in press—due July 1980).

FLAVAHAN, N.A. & McGRATH, J.C. (1980a). Blockade by yohimbine of prazosin-resistant pressor effects of adrenaline in the pithed rat. *Br. J. Pharmac.*, (in press—due July 1980).

FLAVAHAN, N.A. & McGRATH, J.C. (1980b). An analysis of α_1- and α_2-adrenoceptor mediated pressor effects of adrenaline. Communication this meeting.

GILLESPIE, J.S., McLAREN, A. & POLLOCK, D. (1970). A method of stimulating different segments of the autonomic outflow from the spinal column to various organs in the pithed cat and rat. *Br. J. Pharmac.*, **40**, 257–267.

LUCHELLI-FORTIS, M.A. & LANGER, S.Z. (1974). Reserpine-induced depletion of the norepinephrine stores: is it a reliable criterion for the classification of the mechanism of action of sympathomimetic amines? *J. Pharmac. exp. Ther.*, **188**, 640–653.

STANTON, H.C., DUNGAN, K.W. & LISH, P.M. (1965). Amidephrine II: cardiovascular actions of a new sympathomimetic amine. *Int. J. Neuropharmacol.*, **4**, 235–244.

WEITZELL, R., TANAKA, T. & STARKE, K. (1979). Pre- and post-synaptic effects of yohimbine stereoisomers on noradrenergic transmission in the pulmonary artery of the rabbit. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **308**, 127–136.

Differential α-adrenoceptor antagonism by BE 2254 (HEAT) and Prazosin at pre- and post-synaptic receptors in the pithed rat

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BE 2254 'HEAT' (2-[β-(4-hydroxyphenyl) ethyl amino methyl]-tetralone) has been shown to antagonize both peripheral and central α-adrenoceptors (Benthe, Göthert & Tuchinda, 1972; Hoefke & Streller, 1976; Williams, Totaro & Clineschmidt, 1978), and marked hypotensive activity, of central origin, has also been demonstrated (Hoefke & Streller, 1979). Since it is not known at which α-receptors BE 2254 is active, a further classification of the α-adrenoceptor blocking properties of the compound has been made.

Male Wistar rats (250–300 g) were pithed under pentobarbitone (60 mg/kg, i.p.) anaesthesia. Inhibition of the responses induced by continuous electrical stimulation of the thoracic spinal cord (60 V, 0.3 ms, 0.3 Hz) was used as an index of presynaptic agonist activity (Drew, 1976) and the increase in diastolic blood pressure (DBP) used to assess postsynaptic agonist activity. In some experiments DBP was increased by stimulation of the entire sympathetic chain (30 V; 1 ms, 0.1–20 Hz).

Agonist dose–response curves were constructed before, or 15 min after treatment with antagonists (0.01–4.0 mg/kg; i.v.). Cumulative cardioinhibitory responses were obtained to N,N-dimethyl-2-amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (N,N-diMe-6,7-diOHATN) a potent but non-selective α_2-receptor agonist. (Hicks & Cannon: in press) ID_{50} 0.21 (0.19–0.22) μg/kg. Vasoconstrictor response curves were constructed for noradrenaline (NA; a mixed α_1 and α_2 agonist; ED_{50} 0.71 [0.66–0.76] μg/kg); phenylephrine (PE; α_1 agonist; ED_{50} 9.12 [7.76–10.7] μg/kg), or N,N-diMe-6,7-diOHATN (ED_{50} 1.32 [1.21–1.43] μg/kg). The dose of antagonist required to cause a 10 fold shift in the agonist response curve (DR_{10}) is shown in Table 1.

BE 2254 was a competitive antagonist of both PE and NA pressor responses, but, unlike prazosin was equipotent against either agonist. Both prazosin and BE 2254 were considerably less potent antagonists at postsynaptic α_2-receptors, but only BE 2254 antagonized presynaptic α_2-receptors at the heart; prazosin was without effect at this site. BE 2254 did not however, act as a competitive antagonist at these presynaptic sites.

Both prazosin and BE 2254 significantly antagonized ($P < 0.001$) the responses to neuronally released NA, during stimulation of the entire sympathetic chain, further supporting the suggestion that α_1-receptors may have a synaptic location.

Neither the pressor responses to angiotensin II, nor the tachycardia to exogenous NA were antagonized by BE 2254 (4 mg/kg, i.v.) indicating specificity for α-receptors, however BE 2254 enhanced the tachycardia during continuous stimulation of the spinal cord, indicative of presynaptic α_2-receptor antagonism.

In conclusion, BE 2254 was a potent, preferential

Table 1 α-Adrenoceptor blocking potencies of BE 2254 and prazosin in the pithed rat

Agonist	*Post synaptic*			
	BE 2254		*Prazosin*	
	DR_{10} (mg/kg)	Slope*	DR_{10} (mg/kg)	Slope*
NA	0.043 (0.038–0.048)	1.22 (1.00–1.33)	>0.1	—
PE	0.043 (0.039–0.047)	1.15 (1.00–1.22)	0.013 (0.01–0.016)	0.97 (0.91–1.03)
N,N-diMe-6,7-diOHATN	2.03 (1.88–2.18)	1.44† (1.33–1.55)	>1.0	—
Angiotensin II	*Not antagonized*		*Not antagonized*	
	Pre synaptic			
N,N,diMe-6,7-diOHATN	0.011 (0.009–0.014)	0.47† (0.45–0.49)	*Not antagonized*	

Results expressed as geometric mean and 95% confidence limits (n = 5–8).
* Slope of the plot of Log_{10} agonist dose-ratio-1 versus Log_{10} dose of antagonist.
† Significantly different from unity ($P < 0.05$).

antagonist at presynaptic α_2-receptors in the heart, 185× more potent than at postsynaptic vascular α_2-receptors, but 47× less potent at postsynaptic α_2-receptors than at α_1-receptors. In contrast to prazosin, BE 2254 was an equipotent antagonist of the vasoconstrictor responses induced by PE, or exogenous NA, as well as the pressor responses to neuronally released NA.

References

BENTHE, H.F., GÖTHERT, M. & TUCHINDA, P. (1972). Noradrenalin-antagonistische Wirkung Verschiedenel phenyläthylamin- und phenoxyäthylamin-Derivate. *Arzneim. Forsch.*, **22**, 1468–1471.

DREW, G.M. (1976). Effects of α-adrenoceptor agonists and antagonists on pre- and postsynaptically located α-adrenoceptors. *Eur. J. Pharmacol.*, **36**, 313–320.

HICKS, P.E. & CANNON, J.G. (1980). Cardiovascular effects of 2-(N,N-diMethyl) amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene in the pithed rat: differential antagonism by yohimbine and Prazosine. *J. Pharm. Pharmac.* (in press).

HOEFKE, W. & STRELLER, I. (1976). Central blood pressure lowering capacity of α-adrenolytic agents. *Naunyn-Schmiedeberg's Arch. Pharmacol.* **294**, R21.

WILLIAMS, M., TOTARO, J. A. & CLINESCHMIDT, B.V. (1978). Interaction of 2-[β-(4-hydroxyphenyl)ethyl amino-Methyl]-tetralone (BE 2254: 'HEAT') with catecholamine receptors in rat brain membranes. *J. Pharm. Pharmac.*, **30**, 390–391.

HOEFKE, W. & STRELLER, I. (1979). Cardiovascular effect of α-adrenolytic agents: indication of a central presynaptic mode of action. *Naunyn-Schmiedeberg's Arch. Pharmacol.*, **308**, R12.

Heterogeneity of post-junctional purinergic receptors in cardiac and vascular tissue from guinea-pigs

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The cardiovascular effects of adenosine include vasodilatation and cardio-inhibition (Drury & Szent-Gyorgyi, 1929). We have investigated the characteristics of the post-junctional receptors which mediate these effects, using isolated vascular and cardiac tissues from guinea-pigs.

Rings of thoracic aorta and left atria were placed in organ baths containing oxygenated Krebs solution (37°C). Aortic rings were suspended between metal hooks for isometric tension recording, and were contracted with noradrenaline (2×10^{-6} M). This contraction remained stable for at least 30 minutes. Left atria were placed on electrodes and attached to isometric force transducers. They were electrically stimulated (4 Hz 3 ms, threshold voltage 0.5–3 V) and continuously washed with Krebs solution. Electrical stimulation did not activate adrenergic or cholinergic nerve endings in the atria as atenolol (10^{-6} M, $n = 4$) and atropine (10^{-6} M, $n = 6$) had no effect on the amplitude of contraction.

The negative inotropic effect of adenosine on atrial preparations ($n = 8$) and the vascular relaxation evoked by this agonist ($n = 5$) were both significantly potentiated by dipyridamole (10^{-5} M). Similar responses evoked by acetylcholine (atrium), sodium nitrite (aorta) and by the uptake-resistant analogue 2Cl-adenosine (Muller & Paton, 1979) in the atrium and the aorta were unaffected by dipyridamole (10^{-5} M). This indicates that the potentiation of adenosine responses by dipyridamole was due to a specific inhibition of adenosine uptake (Pfleger *et al.*, 1969).

Adenosine was a significantly ($P < 0.001$) more potent negative inotropic agent than A.T.P. on atrial preparations ($pD_2 = 5.41 \pm 0.11$, $n = 8$; $pD_2 = 4.17 \pm 0.16$, $n = 5$, respectively). However, adenosine and A.T.P. were equipotent at relaxing the aorta ($pD_2 = 3.70 \pm 0.17$, $n = 6$; $pD_2 = 3.65 \pm 0.04$, $n = 4$ respectively). Theophylline (10^{-5} to 5×10^{-4} M) was a competitive antagonist of adenosine on atrial preparations ($pA_2 = 5.19 \pm 0.16$, slope = 0.86, $n = 17$). Negative inotropic responses evoked by acetylcholine were unaffected by theophylline (10^{-5} to 5×10^{-4} M). However, in aortic preparations, theophylline (10^{-4} M) had no effect on relaxations evoked by adenosine (pD_2 control $= 3.72 \pm 0.29$; pD_2 in presence of theophylline $10^{-4} = 3.73 \pm 0.14$, $n = 5$). Relaxations evoked by sodium nitrite were also unaltered by theophylline (10^{-4} M). Higher concentrations of theophylline could not be used as they often caused spontaneous relaxation of the aorta.

The specific potentiation of responses to adenosine, by dipyridamole, in aortic and atrial tissue indicates that the receptors that mediate negative inotropism and vascular relaxation both have an extracellular location. However, the difference in the relative

potencies of adenosine and A.T.P. together with the differential blocking effects of theophylline indicates that the purinergic receptors in the two tissues are not of the same type.

References

DRURY, A.N. & SZENT-GYORGYI, A. (1929). The physiological activity of adenine compounds with special reference to their action on the mammalian heart. *J. Physiol. (Lond).*, **68**, 213–237.

MULLER, M.J. & PATON, D.M. (1979). Presynaptic inhibitory actions of 2-substituted adenosine derivatives on neurotransmission in rat vas deferens: effects of inhibitors of adenosine uptake and deamination. *Naunyn-Schmiedebergs' Arch. exp. Path. Pharmac.*, **306**, 23–28.

PFLEGER, K., NIEDERAU, D. & VOLKMER, I. (1969). Ein beitrag zum wirkungsmechanismus von dipyridamol: Hemmung der adenosin aufnahme in erythrocyten durch dipyridamol. *Naunyn-Schmiedebergs' Arch. exp. Path. Pharmac.*, **265**, 118–130.

Anti-proliferative effects of cyclosporin-A and glucocorticosteroids on mitogen-stimulated human blood mononuclear cells

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The immunosuppressive activities of cyclosporin-A and glucocorticosteroids are thought to be partly due to their inhibitory effects on T-lymphocyte proliferation. We examined the effects of dexamethasone, hydrocortisone and cyclosporin-A, alone and in combination, on tritiated-thymidine incorporation into human blood mononuclear cells stimulated by phytohaemagglutinin (PHA), pokeweed mitogen (PWM) and phorbol-12-myristate-13-acetate (PMA) that activate different components of these heterogeneous cell populations (Table 1). Pharmacological concentrations of glucocorticosteroids displaced the PHA and PWM dose–response curves to the right but with the same maximum response being achieved, suggesting inhibition of a competitive nature. In contrast, steroid inhibition of PMA responses was not mitogen dose-dependent and the maximum response was

Table 1

		% Inhibition of control [^3H]-thymidine incorporation at 48–72 h									
	Mitogen (μg/ml)	Phytohaemagglutinin				Pokeweed			Phorbol-Myristate-Acetate		
Drug (μg base/ml)		0.02	0.1	0.5	2.0	0.1	1.0	10.0	0.01	0.1	1.0
Dexamethasone	0.001	36	6	3	−11	7	9	2	7	25	1
	0.01	51	15	−1	−11	35	17	10	36	39	23
	0.1	57	40	9	3	77	49	25	67	62	64
	1.0	54	59	14	5	79	56	14	73	69	71
Hydrocortisone	0.01	27	14	17	−1	−11	2	−6	8	0	−5
	0.1	43	32	17	−3	39	24	4	34	22	16
	1.0	59	46	17	−1	70	44	16	66	57	57
	10.0	54	47	27	24	78	54	33	78	77	78
Cyclosporin-A	0.01	25	−15	8	16	4	10	−4	11	−10	13
	0.1	38	49	37	40	51	47	31	5	5	0
	1.0	60	62	60	57	72	73	67	40	36	33
Dexamethasone + Cyclosporin-A	0.1 0.1	82	85	49	41	83	76	58	75	68	72
Hydrocortisone + Cyclosporin-A	1.0 0.1	79	68	53	45	81	81	56	69	68	68

Each value represents the mean of 9 replicate cultures from 3 separate donors.

clearly depressed, suggesting non-competitive inhibition. The relative activities of dexamethasone and hydrocortisone were consistent with their anti-inflammatory-immunosuppressive dose ratios. In the case of cyclosporin-A, the responses to all mitogens were affected in dose-related but non-competitive manner.

The rank order of sensitivity to inhibition by steroids was PMA $\simeq$ suboptimal PHA (0.02–0.1 μg/ml) and PWM (0.1–1.0 μg/ml) > maximal PHA (0.5–2.0 μg/ml) and PWM (10 μg/ml) responses, while that for cyclosporin-A was PWM $\simeq$ PHA > PMA responses. These differences in sensitivity cannot be attributed simply to changes in the magnitude of the responses since steroid-sensitive PMA responses were of the same order or greater than steroid-resistant PHA and PWM responses. Therefore, these results suggest that a steroid-sensitive mechanism or lymphocyte sub-population may be selectively stimulated by PMA and by low doses of PHA and PWM. The relative resistance to inhibition by cyclosporin-A of PMA-stimulated cells and the ability of cyclosporin-A to inhibit steroid-resistant responses to PHA and PWM might be taken then to indicate that this drug affects a separate sub-population and that PMA activation occurs at or later than the cyclosporin-sensitive stage.

The combined effects of steroids with cyclosporin-A were always greater than either drug alone at the same concentrations. Responses that were susceptible to inhibition by both drugs were not completely inhibited as would have been expected on the basis of simple additive effects, but the level of inhibition by combined treatment was equivalent to the effect of the individual compounds at 10-fold higher concentrations. These results provide some experimental basis for combination therapy in the treatment of reactions involving multiple lymphocyte activation mechanisms.

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References

FAUCI, A.S., DALE, D.C. & BALOW, J.E. (1976). Glucocorticosteroid therapy: Mechanisms of action and clinical considerations. *Ann. Intern. Med.*, **84**, 304–315.

FAUCI, A.S. (1979). Mechanisms of the immunosuppressive and anti-inflammatory effects of glucocorticosteroids. *J. Immunopharm.*, **1**, 1–25.

WHITE, D.J.G., PLUMB, A.M., PAWELEC, G. & BRONS, G. (1979). Cyclosporin A. An immunosuppressive agent preferentially active against proliferating T-cells. *Transplantation*, **27**, 55–58.

WHITE, D.J.G., CALNE, R.Y. & PLUMB, A. (1979). Mode of action of cyclosporin A: a new immunosuppressive agent. *Transplant. Proc.*, **9**, 855–859.