The effects of opioid substances on the secretion of corticotrophin releasing factor in the rat

JULIA C. BUCKINGHAM & J.R. HODGES

Neuroendocrine Unit, Royal Free Hospital School of Medicine, Pond Street, London NW3 2QG

It has been known for many years that morphine influences profoundly the activity of the hypothalamo-pituitary-adrenocortical system (Briggs & Munson, 1955) but its site and mode of action are still not clear. We have studied the effects of morphine, β-endorphin and the enkephalins on the secretion of corticotrophin releasing factor (CRF) by the hypothalamus of the rat. The hypothalami were removed from male Sprague–Dawley animals and incubated exactly as described by Buckingham & Hodges (1977a) in the presence and absence of the opioid substances. The CRF content of the hypothalamic tissue and the incubation media were determined using a method which depends upon the ability of the hypothalamic hormone to stimulate segments of anterior pituitary tissue to secrete corticotrophin (Buckingham & Hodges, 1977b). None of the drugs studied influenced directly the corticotrophic activity of adenohypophysial tissue *in vitro*. Met-enkephalin (10^{-10}–10^{-6} M) and leu-enkephalin (10^{-8} M) increased both the CRF content of rat hypothalami and the media in which they were incubated. Morphine also stimulated hypothalamic CRF production in low concentrations (10^{-10}–10^{-6} M) although in higher concentrations (10^{-5}–10^{-4} M) its effects were less marked. The stimulatory effects of the enkephalins and of morphine were antagonized not only by naloxone (10^{-8} M) but also by β-endorphin (10^{-6}–10^{-4} M).

The results are in accord with the suggestion (Gibson, Ginsburg, Hall & Hart, 1979) that opioid substances influence the functional activity of the hypothalamo-pituitary-adrenocortical system by controlling the secretion of CRF at the hypothalamic level.

We are grateful to Dr. S Wilkinson and Dr. I. Briggs, Wellcome Research Laboratories, for enkephalins, to Dr. J. Vernikos-Danellis for β-endorphin, to the World Health Organization for lllrd corticotrophin I.W.S., to the Medical Research Council for financial support and to Mrs Janet Dobson for excellent technical assistance.

References

BRIGGS, F.N. & MUNSON, P.L. (1955). *Endocrinology*, **57**, 205–219.

BUCKINGHAM, J.C. & HODGES, J.R. (1977a). *J. Physiol.*, **272**, 469–479.

BUCKINGHAM, J.C. & HODGES, J.R. (1977b). *J. Endocr.*, **72**, 187–193.

GIBSON, A., GINSBURG, M., HALL, M. & HART, S.L. (1979). *Br. J. Pharmac.*, **65**, 139–146.

Potassium stimulated depletion of enkephalin in rat hypothalamus and its alteration by adrenocorticotrophin and corticosterone

S.L. HART & I. KITCHEN

Department of Pharmacology, Chelsea College (University of London) London SW3 6LX

Experimental alteration of hypothalamus-pituitary-adrenal (HPA) axis activity can produce changes in the enkephalin content of rat hypothalamus (Gibson, Ginsburg, Hart & Kitchen, 1979). We now describe an *in vitro* system employing an indirect method of measuring enkephalin release, similar to that of Hughes, Kosterlitz & Sosa (1978), and its modification by adrenocorticotrophic hormone (ACTH) and corticosterone.

Hypothalamic slices (0.47 mm) were incubated with Krebs solution (8 ml) at 37°C and constantly aerated with 95% O_2/5% CO_2 for 1 hour. Enkephalin content of the slices was then measured by bioassay against met-enkephalin on the mouse vas deferens after extraction of the tissues in HCl (0.1 M) and purification on Amberlite XAD-2 (Hughes, Kosterlitz & Smith, 1977). Depletion of enkephalin from the tissue was achieved by incubation in Krebs solution containing 50 mM KCl.

The depleting effect of K^+ increased with time and a significant fall was seen after stimulation for 1 h (957 ± 65 vs 555 ± 36 ng/g met-enkephalin equivalents, $P < 0.001$). At this time the content of unstimulated slices was significantly higher than that measured after 10 min, indicating enkephalin production during incubation (957 ± 65 vs 715 ± 34 ng/g, $P < 0.01$). The presence of cycloheximide (0.1 mM) in the incubation had no effect on the enkephalin content of unstimulated or K^+-stimulated slices, in contrast to the observations in guinea-pig myenteric plexus (Hughes *et al.*, 1978). Assuming that this concentration of cycloheximide would inhibit *de novo* synthesis, the results suggest that there may be a large

enkephalin precursor store in the hypothalamus. In the absence of Ca^{2+}, resting levels of enkephalin were unchanged but there was no depletion in slices incubated for 1 h in Ca^{2+}-free Krebs containing K^+ (50 mM).

The presence of corticosterone in normal Krebs reduced enkephalin content. Concentrations of corticosterone used were 58 nM, 580 nM and 5.8 μM and with each there was significant depletion ($P < 0.05$) of enkephalin by about 30% which was apparently maximal at the lowest concentration. In the presence of corticosterone, K^+ (50 mM) depleted enkephalin content of slices but only to the level seen in the absence of corticosterone. However, if the slices had been depleted by as much as possible during incubation with K^+ (50 mM) any additional effect of corticosterone would be masked.

Incubation for 1 h with $ACTH_{1-24}$ (1 nM) significantly increased the enkephalin content by approximately 20%; 10 nM caused a significant reduction of approximately 30% which was not greater when the $ACTH_{1-24}$ concentration was increased to 100 nM. In slices incubated in the presence of K^+ (50 mM), at all the concentrations of $ACTH_{1-24}$ or corticosterone used, the enkephalin content was not different from that of slices incubated with high K^+ alone.

Though it is not possible to interpret these results as direct effects on enkephalin release, it is concluded that components of the HPA axis modify enkephalin turnover *in vitro* as well as *in vivo*.

I.K. is an M.R.C. Student.

References

GIBSON, A., GINSBURG, M., HART, S.L. & KITCHEN, I. (1979). Enkephalin content in rat hypothalamus—effect of adrenalectomy, laparotomy and corticosteroid treatment. *Br. J. Pharmac.*, **66**, 130P.

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

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

Antagonism of the excitatory action of ACTH (1–24) by D-phe^7 ACTH (4–10) on single neurones in the rat medulla

J.P. GENT & J.R. NORMANTON

Department of Pharmacology, University of Leeds

The question of a central role for adrenocorticotrophic hormone (ACTH) has been raised over recent years by several workers. For example, certain analogues of ACTH have been shown to produce behavioural effects (de Wied & Gispen, 1977: File, 1979). ACTH (1–39), ACTH (1–24), ACTH (1–10), ACTH (4–10) and ACTH (4–7) delay the extinction of active avoidance behaviour. If the L-phenylalanine residue in position seven is replaced by D-phenylalanine, however, the extinction is facilitated (de Wied, Witter & Greven, 1975).

We have investigated the effects of certain ACTH analogues on the activity of single neurones in the brainstem, where presence of ACTH-like immunoreactivity has been observed (Watson, Richard & Barchas, 1978).

Experiments were performed on rats (200–400 gm) anaesthetized with urethane (1.5 g/kg). Single units were recorded extracellularly in the medullary reticular formation (1–3 mm rostral to the obex, 0.5–1.0 mm lateral to the midline and to a depth of 2.5 mm from the upper surface of the brainstem). Drugs were applied microiontophoretically from five or six-barrelled micropipettes, one barrel of which contained 1 M NaCl for continuous current compensation or current control.

ACTH (1–24), applied with currents of 0–120 nA, increased the firing rate of the majority of cells studied, in a dose-dependent manner. The response was slow in onset, usually reaching a plateau within 90 seconds. On termination of the ejection current, the firing rate returned to its resting level over a similar period. ACTH (4–7), ACTH (4–10) and D-phe^7 ACTH (4–10) usually produced no change in firing rate when applied with currents of up to 200 nA for several minutes.

On some cells tested, a continuous application of D-phe^7 ACTH (4–10) produced a marked reduction in the size of the response to ACTH (1–24), while matching responses to acetylcholine and L-glutamate were unaffected. The effect developed over a period of up to 4 min, and, on termination of the application of D-phe^7 ACTH (4–10), responses to ACTH (1–24) recovered within a period of 10 minutes.

This specific and reversible antagonism by D-phe^7 ACTH (4–10) of the excitatory action of ACTH (1–24) may provide an explanation for the opposing behavioural effects of these two analogues.

We are grateful to Dr. J. J. de Ridder of Organon, Oss, for supplying the ACTH analogues. JRN is an MRC scholar.

References

DE WIED, D. & GISPEN, W.H. (1977). Behavioural effects of peptides. In: *Peptides in neurobiology*. (Ed. H. Gainer) pp. 402–418. Plenum Press, N.Y.

DE WIED, D., WITTER, A. & GREVEN, H.M. (1975). Behaviourally active ACTH analogues. *Biochem. Pharmacol.*, **24**, 1463–1468.

FILE, S.E. (1979). Effects of ACTH (4–10) in the social interaction test of anxiety. *Brain Research.*, **171**, 157–160.

WATSON, S.J., RICHARD, C.W. III., BARCHAS, J.D. (1978). Adrenocorticopin in rat brain: Immunocytochemical localisation in cells and axons. *Science.*, **200**, 1180–1182.

Behavioural effects of central and peripheral injection of two synthetic analogues of TRH

A.R. GREEN, D.J. HEAL, AMAL SABBAGH & M.B.H. YOUDIM

MRC Unit and University Department of Clinical Pharmacology, Radcliffe Infirmary, Oxford, OX2 6HE, and Department of Pharmacology, Technion, Haifa, Israel

TRH (pyroglutamyl-histidyl-prolyl amide) produces behavioural excitation and increased locomotor activity following either peripheral or central injection (Miyamoto & Nagawa, 1977; Heal & Green, 1979). Behavioural data suggested that TRH released dopamine in the n. accumbens but not n. caudatus (Heal & Green, 1979), and recent biochemical evidence supports this (Kerwin & Pycock, 1979). Replacement of the pyroglutamyl residue of TRH by an orotyl (CG 3509) or a 6-methyl-5-oxo-thiomorpholinyl-3-carbonyl (CG 3703) group produced compounds resistent to peptidases but which possessed both the endocrinological and some of the behavioural activity of TRH (Friderichs, Schwertner, Herrling, Gunzler & Flohé, 1979). The behavioural effects of these two compounds following peripheral and central administration to rats have now been studied.

Injection of CG 3509 (5 μg bilaterally) or CG 3703 (5 μg bilaterally) into the n. accumbens caused locomotor activity and other behavioural changes (Table 1). The two analogues were approximately equipotent and the behavioural changes observed were stimilar to those produced by injection of TRH, but much greater in magnitude and duration (Table 1). Administration of haloperidol (2.5 μg bilaterally) into the n. accumbens 15 min before either CG 3509 (5 μg bilaterally) or CG 3703 (5 μg bilateraly) reduced the locomotor response by about 50%.

Peripheral injection of CG 3509 (5 mg/kg i.p.) or CG 3703 (5 mg/kg i.p.) also produced prolonged locomotor activity and behavioural excitation, similar to the short-lasting changes observed after injection of TRH (10 mg/kg i.p.; Table 1). Pretreatment with haloperidol (2 mg/kg i.p.) 30 min before TRH (10 mg/kg), CG 3509 (5 mg/kg) or CG 3703 (5 mg/kg) blocked these effects.

Table 1 Effect of intraperitoneal or intra-accumbens of TRH and the two synthetic analogues CG 3509 and CG 3703 on rat locomotor activity

	Intra-accumbens injection		*Intraperitoneal injection*	
Drug	*Dose*	*Activity counts*	*Dose*	*Activity counts*
Saline	(1 μl bilaterally)	598 ± 62 (5)	(0.5 ml)	781 ± 110 (6)
TRH	(10 μg bilaterally)	956 ± 130 (4)*	(10 mg/kg)	1652 ± 192 (6)**
CG 3509	(5 μg bilaterally)	3081 ± 225 (5)†	(5 mg/kg)	2852 ± 192 (6)†
CG 3703	(5 μg bilaterally)	3219 ± 410 (5)†	(5 mg/kg)	3150 ± 281 (6)†

The results are the mean ± s.e. mean of the total number of movements in the 90 min period following injection of drug for a single rat in intra-accumbens injection experiments, or the 60 min period following injection of drug for groups of three rats in intraperitoneal injection experiments; both after discounting the first 5 min period. The number of observations are shown in brackets. Activity was measured using LKB Animex activity meters; sensitivity and tuning 30 μA.

* Different from saline $P < 0.05$, ** different from saline $P < 0.005$, † different from saline and TRH $P < 0.05$. Results were analysed using a Student's unpaired '*t*' test. Intra-accumbens TRH data taken from Heal & Green (1979).

Intraperitoneal injection of CG 3509 (5 mg/kg) or CG 3703 (5 mg/kg) into unilateral nigro-striatal lesioned rats produced behavioural excitation but no circling. TRH also fails to produce circling behaviour (Heal & Green, 1979), suggesting that these compounds do not release dopamine in the n. caudatus.

Preliminary data suggests therefore that CG 3509 and CG 3703 mimic TRH and release dopamine in the n. accumbens but not the n. caudatus. However, the resistance of these compounds to enzymatic degradation potentiates their behavioural effects.

This work was supported in part by a grant from Grünenthal GmbH, Aachen, to M.B.H.Y.

References

FRIDERICKS, E., SCHWERTNER, E., HERRLING, S., GUNZLER, W.A. & FLOHE, L. (1979). Activity of thyroliberin analogues with a modified pyroglutamyl residue on the central nervous system. *Hoppe-Selers Z. Physiol. Chem.*, **360**, 1146.

HEAL, D.J. & GREEN, A.R. (1979). Administration of thyrotropin releasing hormone (TRH) to rats releases dopamine in n. accumbens but not n. caudatus. *Neuropharmacology* **18**, 23–32.

KERWIN, R.W. & PYCOCK, C.J. (1979). Thyrotropin releasing hormone stimulates release [^{3}H]-dopamine from slices of rat nucleus accumbens *in vitro*. *Br. J. Pharmac.*, **67**, 323–325.

MIYAMOTO, M. & NAGAWA, Y. (1977). Mesolimbic involvement in the locomotor stimulant action thyrotropin-releasing-hormone (TRH) in rats. *Eur. J. Pharmac.*, **44**, 143–152.

Differential effects of sodium salicylate and BW755C on prostaglandin formation in inflammatory exudates and the gastro-intestinal tract

K.E. EAKINS, G.A. HIGGS & B.J.R. WHITTLE

Dept. Prostaglandin Research, Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

Inhibition of prostaglandin biosynthesis has been implicated as a mechanism involved in both the anti-inflammatory actions and the gastro-intestinal side-effects of aspirin-like drugs (Vane, 1971). Prostaglandin E_2 (PGE_2) and prostacyclin (PGI_2), cyclo-oxygenase products, found in carrageenin-induced inflammatory exudates (Higgs & Salmon, 1979) have pro-inflammatory actions, whereas these prostanoids can exert protective actions on the gastric mucosa and small intestine (Robert, 1974; Whittle & Boughton-Smith, 1979). To reduce the gastro-intestinal damage caused by non-steroid anti-inflammatory drugs it would thus be desirable to inhibit prostaglandin production only at the site of inflammation and not in the gastro-intestinal tract. We have, therefore, investigated whether such differential inhibition of cyclo-oxygenase can be achieved *in vivo* in the rat and have correlated this with gastric toxicity.

Inflammatory exudates were induced and collected by the subcutaneous implantation of carrageenin-impregnated polyester sponges (Higgs, Flower & Vane, 1979). After 24 h the prostaglandin-like activity in the sponge was extracted and bioassayed on the rat superfused stomach-strip in terms of PGE_2 equivalents (the major prostaglandin product in the exudate). The *ex vivo* generation of prostacyclin, the predominant prostaglandin in the gastric mucosa, was determined as previously described (Whittle, 1978). Strips (0.2–0.4 g) of gastric mucosa or ileum from which the mesentary had been removed were incubated in buffer (50 mM Tris: pH 8.4, 1 ml) by vortex mixing (1 min 22°C). The supernatant was immediately tested for its ability to inhibit platelet aggregation and this prostacyclin-like activity, characterised as before, was assayed against authentic prostacyclin.

Drugs were administered orally to groups of six rats, three times over a 24 h period in anti-inflammatory doses previously determined to cause 50–70% inhibition of rat paw oedema. The recently described dual lipoxygenase-cyclo-oxygenase inhibitor, BW755C (3 amino-l[m-trifluoromethyl)-phenyl]-2-pyrazoline) was administered in doses ten-fold greater than those required for inhibition of rat paw oedema (Higgs, *et al.*, 1979). Aspirin (200 mg/kg), indomethacin (4 mg/kg) and ketoprofen (4 mg/kg) significantly ($P < 0.01$) reduced prostaglandin levels in the implanted sponge (by $83 \pm 5\%$, $98 \pm 2\%$ and $98 \pm 1\%$ respectively; mean ± s.e. mean). Likewise these compounds reduced prostacyclin formation in the gastric mucosa (by $96 \pm 2\%$, $90 \pm 2\%$ and $84 \pm 3\%$ respectively) and ileum (by $87 \pm 6\%$, $91 \pm 1\%$ and $73 \pm 11\%$ respectively). In contrast, sodium salicylate (200 mg/kg) and BW755C (100 mg/kg) caused a significant ($P < 0.01$) inhibition of prostaglandins in the sponge inflammatory exudate (by $85 \pm 3\%$ and $86 \pm 9\%$) yet had no significant ($P > 0.05$) effect on *ex vivo* prostacyclin production in either the gastric mucosa or ileum. In addition, sodium salicylate and BW755C

caused markedly ($P < 0.05$) less gastric damage, assessed as previously described (Whittle & Boughton-Smith, 1979) than aspirin, indomethacin or ketoprofen when administered orally three times over 24 h in the above doses. These latter findings support the relationship between production of gastric erosions and the inhibition of mucosal prostacyclin production.

Whereas *in vitro* studies have shown differential sensitivities between the cyclo-oxygenase extracted from such tissues as spleen and brain to drugs such as paracetamol (Flower & Vane, 1974), the present findings clearly indicate that sodium salicylate and BW755C can selectively inhibit prostaglandin production *in vivo* in the inflammatory exudate and not in the gastro-intestinal tract. It is possible that cyclo-oxygenase activity at the site of inflammation is highly sensitive to inhibition by these two compounds *in vivo*. The development of non-steroid anti-inflammatory drugs which do not prevent prostaglandin production by the gastro-intestinal tract and exhibit reduced gastro-intestinal toxicity thus appears to be a feasible proposition.

We thank Mr. K. Mugridge and Mrs. J. Walker for technical assistance and Dr. C. V. Denyer and Dr. F.C. Copp for preparing BW755C.

References

FLOWER, R.J. & VANE, J.R. (1974). Inhibition of prostaglandin biosynthesis. *Biochem. Pharmacol.*, **23**, 1439–1450.

HIGGS, G.A. & SALMON, J.A. (1979). Cyclo-oxygenase products in carrageenin-induced inflammation. *Prostaglandins*, **17**, 737–746.

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

ROBERT, A. (1974). Effects of prostaglandins on the stomach and the intestine. *Prostaglandins*, **6**, 523–532.

VANE, J.R. (1971). Inhibition of prostaglandin biosynthesis as a mechanism of action of aspirin-like drugs. *Nature, (New Biol.)*, **231**, 232–233.

WHITTLE, B.J.R. (1978). Potential endogenous inhibitor of prostaglandin synthetase in plasma: failure to inhibit cyclo-oxygenase in platelets and the gastric mucosa. *J. Pharm. Pharmacol.*, **30**, 467–468.

WHITTLE, B.J.R. & BOUGHTON-SMITH, N.K. (1979). 16-phenoxy prostacyclin analogues; Potent selective anti-ulcer compounds. In: *Prostacyclin*, eds. J.R. Vane & S. Bergstrom, pp. 159–170, New York, Raven Press.

The influence of the oestrous cycle on the metabolism of exogenous arachidonate in rat isolated lung

Y.S. BAKHLE & J.T. ZAKRZEWSKI

Department of Pharmacology, Institute of Basic Medical Sciences, Royal College of Surgeons of England, Lincoln's Inn Fields, London WC2A 3PN

Many blood borne substrates are metabolized on passage through the pulmonary circulation. Monoamine and peptide metabolism occurring in lung is affected by the oestrous cycle (Bakhle & Ben-Harari, 1979a, b). Arachidonic acid is also metabolized by lung and we have therefore investigated the metabolism of exogenous [^{14}C]-arachidonate in isolated lungs from female rats at defined stages of the 4-day oestrous cycle.

Virgin female rats kept under controlled lighting conditions were allowed to cycle normally and the stages checked by vaginal smears. Lungs were removed from animals after smearing and perfused via the pulmonary circulation with Krebs solution at 8 ml/min. Exogenous [^{14}C]-arachidonate was infused (40 μM; 2.5 μCi per infusion) for 3 min at 0.1 ml/min into the Krebs perfusate entering the lung. The effluent was collected during and after the infusion for 10 min and, following extraction, analysed by t.l.c. (Al-Ubaidi & Bakhle, 1979). The total radioactivity in the 10 min collection was also measured. Radioactivity remaining in the lung after the 10 min perfusion was extracted by homogenization and analysed (Jose & Seale, 1979) into phospho- and neutral lipid and free fatty acid fractions.

Results were obtained from animals during all four stages of the cycle but the differences were most obvious ($P < 0.05$) between rats during oestrus ($n = 4$) and the immediately preceding stage, pro-oestrus ($n = 6$). During oestrus, the total effluent radioactivity was higher (11 ± 2% of total infused vs. 6 ± 1%) and of that radioactivity, less was present as cyclo-oxygenase products (thromboxanes, prostaglandins and their metabolites), i.e. 14 ± 2% of effluent radioactivity vs. 27 ± 4%. For the retained radioactivity, during oestrus the proportion in phospholipid was lower (27 ± 5% vs. 43 ± 3%) and that in the free acid fraction higher (49 ± 6% vs. 34 ± 3%) than during pro-oestrus.

The differences observed in these experiments are small but an assessment of their physiological significance requires a more detailed analysis of the various

labelled metabolite fractions. These results suggest that arachidonate metabolism in lung is susceptible to modulation by physiological alterations in hormone levels.

This work was supported by the M.R.C.

References

AL-UBAIDI, F. & BAKHLE, Y.S. (1979). The fate of exogenous arachidonic acid in guinea-pig isolated lungs. *J. Physiol.*, **295**, 445–455.

BAKHLE, Y.S. & BEN-HARARI, R.R. (1979a). Effects of oestrous cycle and exogenous ovarian steroids on 5-hydroxytryptamine metabolism in rat lung. *J. Physiol.*, **291**, 11–18.

BAKHLE, Y.S. & BEN-HARARI, R.R. (1979b). Metabolism of angiotensin and bradykinin in rat isolated lungs during the oestrous cycle. *J. Physiol.*, **292**, 38–39P.

JOSE, P.J. & SEALE, J.P. (1979). Incorporation and metabolism of ^{14}C-arachidonic acid in guinea-pig lungs. *Br. J. Pharmac.*, **67**, 519–526.

Generation of chemokinetic activity from rat polymorphonuclear leucocytes treated with calcium ionophore A23187

M.A. BRAY, A.W. FORD-HUTCHINSON & M.J.H. SMITH

Biochemical Pharmacology Research Unit, Department of Chemical Pathology, King's College Hospital Medical School, Denmark Hill, London, SE5 8RX

Treatment of human polymorphonuclear leucocytes (PMNs) with calcium ionophore (A23187) induces the release of products of the lipoxygenase pathways of arachidonic acid (AA) metabolism (Borgeat & Samuelsson, 1979; Stenson & Parker, 1979). We have investigated the release of chemokinetic activity from rat PMNs treated with A23187 in an attempt to correlate this phenomenon with release of lipoxygenase pathway products.

Rat cell suspensions (>85% PMNs) were prepared from peritoneal exudates obtained 24 h after the injection of 12% sodium caseinate. The cells were washed and incubated with stirring (37°C, 800 rev/min) at a concentration of 1×10^7 cells/ml in Eagle's minimum essential medium buffered to pH 7.4 in the presence of A23187 (10^{-5} M). Prior to the addition of A23187 the cells were pre-incubated for 5 min either with or without drugs. The cells were removed by centrifugation and aliquots of the supernatants assayed for chemokinetic activity towards human PMNs using an agarose micro-droplet assay (Smith & Walker, 1980).

Following incubation of rat PMNs with A23187, dilutions of the supernatants from 1:100 to 1:1000 caused chemokinesis of human PMNs. Maximal release of chemokinetic activity occurred after 4 minutes. The activity could be extracted into diethyl ether following acidification of the medium and could be recovered from silica gel thin layer chromatography plates in an area with a Rf value intermediate between that of arachidonic acid and PGE_2 (solvent system; hexane:ethyl acetate:acetic acid, 1:1:0.005). Production of chemokinetic activity was inhibited by nordihydroguaiaretic acid (10^{-4} to 10^{-7} M), 5, 8, 11, 14 eicosatetraynoic acid (10^{-4} to 10^{-6} M), BW 755C (10^{-4} M) and benoxaprofen (10^{-4} M), all compounds known to inhibit lipoxygenase pathways. Production of chemokinetic activity was partially inhibited by 10^{-4} M indomethacin (51% inhibition) and 5×10^{-4} aspirin (36% inhibition) but was not significantly inhibited by 10^{-4} M phenylbutazone, naproxen for flurbiprofen. There was no correlation between inhibition of production of chemokinetic activity and production of thromoxane B_2 by rat PMNs (Ford-Hutchinson, Bray & Smith, 1979).

The results suggest that PMNs release a product of the lipoxygenase pathway of AA metabolism with chemokinetic activity towards PMNs and that release of this activity by A23187 may be a useful model for studying the effects of pharmacological agents on these pathways.

We are grateful to the Arthritis and Rheumatism Council for financial support (M.A.B.).

References

BORGEAT, P. & SAMUELSSON, B. (1979). Arachidonic acid metabolism in polymorphonuclear leukocytes: Effects of ionophore A23187. *Proc. Nat. Acad. Sci.* **76**, 2148–2152.

FORD-HUTCHINSON, A.W., BRAY, M.A. & SMITH, M.J.H. (1979). The aggregation of rat neutrophils by arachidonic acid: a possible bioassay for lipoxygenase activity. *J. Pharm. Pharmacol.*, **12**, 868–869.

SMITH, M.J.H. & WALKER, J.R. (1980). The effects of some antirheumatic drugs on an *in vitro* model of human polymorphonuclear leucocyte chemokinesis. *Brit. J. Pharmacol.* In press.

STENSON, W.F. & PARKER, C.W. (1979). Metabolism of arachidonic acid in ionophore-stimulated neutrophils. *J. Clin. Invest.* **64**, 1457–1465.

Studies on prostacyclin formation by the rat pregnant myometrium

K.E.H. EL TAHIR & K.I. WILLIAMS

Department of Pharmacology, School of Pharmacy and Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY

In the rat pregnant uterus, the myometrium is the main source of an antiaggregatory material which closely resembles prostacyclin (PGI_2) (Williams, Dembinska-Kiec, Zmuda & Gryglewski, 1978). We have carried out further experiments to find the optimal incubation conditions and to identify the released material as PGI_2.

Wistar rats were killed on days 20–22 of pregnancy, after removing the uterine contents the areas of myometrium lying beneath the placentae were excised and discarded. Decidual tissue was separated from the myometrium by scraping (Williams & El Tahir, 1980). Myometrial tissue was then divided into samples which were incubated (25% w/v) in ungassed Kreb's solution (pH8) or Tris buffer (pH7.4 or pH8) at 20°C or 37°C. Samples were chopped finely and after incubation the PGI_2-like activity in the media was determined by comparison of the inhibition of platelet aggregation induced by adenosine disphosphate with that produced by authentic PGI_2. Statistical significance was calculated using the Student '*t*' test.

In Kreb's solution or Tris buffer at pH7.4 or pH8 maximal production of antiaggregatory activity had occurred within 15 min after incubation at 20°C (3.2 ± 0.22 ng/mg PGI_2 equivalents; s.e. mean; $n = 4$) but within 3 min at 37°C (2.06 ± 0.14 ng/mg, $n = 4$), these values are significantly different ($P < 0.05$). However, gas chromatographic—mass spectrometric analysis of the samples showed the 6-oxo-$PGF_{1\alpha}$ content to be similar in both cases. Hence at 37°C maximal production occurs more rapidly but so does hydrolysis of the antiaggregatory material. Thus 20°C was the preferred incubation temperature; selection of the pH of the incubation medium was then critical as the anti-aggregatory material was stable for at least 20 min at pH8 but disappeared completely within this time when incubated at pH7.4. The alkaline stability of the activity produced was further demonstrated by maintaining the incubation media at pH12 for 60 min at 4°C and after neutralization no loss of antiaggregatory potency was noted, similarly for authentic PGI_2. However, the antiaggregatory activity of PGD_2 and PGE_2 were completely abolished.

Several drugs known to inhibit arachidonic acid metabolism were then investigated for their effects on the activity released by the myometrial tissue. Preincubation of myometrial samples with inhibitory drugs at 20°C was found to be unsatisfactory as large concentrations and long incubation periods were required. However, much smaller doses of drug could be used if the myometrium was preincubated with the inhibitor for 10 min at 37°C prior to chopping and subsequent incubation. Indomethacin (30 μg/ml); 5,8,11,14-eicosatetraynoic acid (TYA) (30 μg/ml); mepacrine (400 μg/ml); 15-hydroperoxyarachidonic acid (30 μg/ml) and tranylcypromine (320 μg/ml) all significantly reduced the output of the antiaggregatory material. Surprisingly hydrocortisone (5–20 μg/ml) was found to increase PGI_2 release in a dose-dependent fashion.

Incubation of myometrial samples with arachidonic acid (5–20 μg/ml) or phospholipase A_2 (0.5–1 iu/ml) significantly increased the release of antiaggregatory material.

These results provide further evidence that the rat pregnant myometrium is a rich source of the arachidonic acid metabolite PGI_2.

We thank the Sudanese Government for support; the Upjohn Company for prostaglandins; Wellcome Laboratories for PGI_2 and Dr. C. N. Hensby for performing the GC-MS analysis.

References

WILLIAMS, K.I., DEMBINSKA-KIEC, A., ZMUDA, A. & GRYGLEWSKI, R.J. (1978). Prostacyclin formation by myometrial and decidual fractions of the pregnant rat uterus. *Prostaglandins*, **15**, 343–350.

WILLIAMS, K.I. & EL TAHIR, K.E.H. (1980). Spatial and temporal variations in prostacyclin production by the rat pregnant uterus. *Adv. in Prostaglandin & Thromboxane Res.*, **8**, 1413–1417.

Oxytocic drugs stimulate prostacyclin release from the rat pregnant myometrium

K.E.H. EL TAHIR & K.I. WILLIAMS

Department of Pharmacology, School of Pharmacy & Pharmacology, University of Bath, Claverton Down, Bath BA2 7AY

Myometrial tissue has been shown to be the major source of prostacyclin (PGI_2) in the rat pregnant uterus (Williams, Dembinska-Kiec, Zmuda & Gryglewski, 1978) and production is stimulated in the presence of oxytocin or bradykinin (El Tahir & Williams, 1979). The present report is an extension of these findings.

Wistar rats were killed on days 18–20 of pregnancy; after removal of the uterine contents, decidual tissue and areas of myometrium which lay beneath the placentae were removed and discarded. Samples of the remaining myometrial tissue were suspended in Kreb's solution (25% w/v). Tissue samples were preincubated at 37°C for 20 minutes. In control samples this preincubation was carried out without drug addition; in test samples the relevant oxytocic drug was added to the medium after 10 min and the incubation conducted for a further 10 minutes. Some samples were preincubated with the phospholipase inhibitor mepacrine (320 μg/ml) for 10 min before addition of oxytocic drugs. The following drugs were used: angiotensin II (10 μg/ml); ergometrine (10 μg/ml); oxytocin (10 mu/ml); bradykinin (20 μg/ml) and $PGF_{2\alpha}$ (20 μg/ml). After the preincubation period the tissue samples were chopped finely and incubated for a further 15 minutes at 20°C. Incubation fluid was then aspirated and the prostacyclin content estimated by inhibition of aggregation of rabbit platelet-rich plasma (Williams *et al.*, 1978). Levels of statistical significance were calculated using a paired '*t*' test.

Angiotensin II caused a significant increase in myometrial PGI_2 generation from 1.83 ± 0.31 (control) to 3.34 ± 0.54 ng/mg wet weight of tissue (mean ± s.e. mean; $P < 0.01$; $n = 4$). Ergometrine also significantly increased PGI_2 generation from 1.87 ± 0.23 to 3.64 ± 0.20 ng/mg ($P < 0.02$; $n = 4$). Similar increases were also seen with bradykinin, PGI_2 production rising from 1.43 ± 0.03 to 2.82 ± 0.20 ng/mg ($P < 0.05$; $n = 4$) and oxytocin, 1.13 ± 0.13 to 2.18 ± 0.13 ng/mg ($P < 0.05$; $n = 4$). Although $PGF_{2\alpha}$ caused a small increase in PGI_2 synthesis from 1.63 ± 0.23 to 1.81 ± 0.21 ng/mg ($n = 5$) this was not significant. The influence of mepacrine was studied in all experiments and was found to significantly reduce the drug-stimulated release of PGI_2 from the myometrium (range 66–90%).

The results suggest that some but not all drugs which are potent uterine stimulants are also potent stimulants of PGI_2 production. This action appears to be mediated via activation of phospholipase A_2 which increases precursor availability. The stimulated PGI_2 generation may play a role in modulating the actions of such drugs in the uterus.

We thank the Sudanese Government for support, the Upjohn Company for prostaglandins, Wellcome Laboratories for PGI_2 and Sandoz Ltd. for oxytocin.

References

EL TAHIR, K.E.H. & WILLIAMS, K.I. (1979). Myometrial prostacyclin synthesis—a new factor in pregnancy in the rat? *J. Physiol. (Lond.)*, **296**, 86P.

WILLIAMS, K.I., DEMBINSKA-KIEC, A., ZMUDA, A. & GRYGLEWSKI, R.J. (1978). Prostacyclin formation by myometrial and decidual fractions of the pregnant rat uterus. *Prostaglandins*, **15**, 343–350.

The effects of prostacyclin on infarct flow and epicardial ST-segment elevation in anaesthetized dogs

SUSAN J. COKER & J.R. PARRATT

Department of Physiology and Pharmacology, University of Strathclyde, Glasgow G1 1XW, and of Surgery, University of Glasgow (Western Infirmary)

Prostacyclin has been shown to be a potent vasodilator in both the systemic and coronary circulations (Dusting, Chapple, Hughes, Moncada & Vane, 1978) and has also been shown to have a protective effect in acute myocardial ischaemia in cats (Ogletree, Lefer, Smith & Nicolaou 1979). We have investigated the effects of prostacyclin on blood flow and on epicardial electrocardiograms in the acutely ischaemic myocardium in greyhounds anaesthetised with chloralose.

In one group of dogs the left anterior descending coronary artery was permanently ligated and infarct flow was measured, using a xenon clearance technique (Marshall, Parratt & Ledingham, 1974), at 20–30 min intervals commencing 1 h after ligation. After establishing consistent control flow values, prostacyclin was administered as an intravenous infusion starting

5 min prior to the measurement of haemodynamics and infarct flow. Prostacyclin (0.01 to 1 μg kg^{-1} min^{-1}) caused dose dependent decreases in arterial blood pressure (BP), left ventricular (LV) dP/dt_{max}, peripheral coronary pressure (PCP) and infarct flow. In a dose of 0.5 μg kg^{-1} min^{-1} prostacyclin significantly ($P < 0.05$) reduced mean BP (from 110 ± 10 to 76 ± 13 mmHg), LV dP/dt_{max} (from 1630 ± 320 to 1040 ± 170 mmHg s^{-1}), mean PCP (from 26 ± 2 to 19 ± 1 mmHg) and infarct flow (15 ± 3 to 7 ± 3 ml 100 g^{-1} min^{-1}). Prostacyclin caused no significant changes in heart rate, pulmonary artery pressure, LV end-diastolic pressure, cardiac output or in the oxygen tension or content of coronary sinus blood or of blood sampled from a local coronary vein draining the ischaemic area. Oxygen consumption in the ischaemic area was significantly reduced (e.g. from 4.10 ± 0.97 to 2.13 ± 0.61 ml/100 g by 0.1 μg kg^{-1} mim^{-1} prostacyclin). There was a significant correlation ($r = 0.82$; $P < 0.001$) between PCP and infarct flow. These results suggest that the decrease in infarct flow caused by prostacyclin results from the decrease in coronary perfusion pressure.

In a second group of dogs, a rubber pad holding nine epicardial electrodes was sutured to the myocardium (Marshall & Parratt, 1977). Short (3 min) occlusions were carried out at 20–30 min intervals and ST-segment elevation was measured in each of the nine leads before, and after 1, 2, and 3 min of occlusion. Prostacyclin 0.1 and 0.5 μg kg^{-1} min^{-1}, infused intravenously commencing 5 min prior to occlusion, significantly reduced (e.g. by 20% and 38% respectively at 1 min) the changes in ST-segment elevation resulting from coronary artery occlusion.

Although i.v. prostacyclin reduces blood flow in the ischaemic area, oxygen content is unaltered and oxygen consumption is reduced which suggests that the reduction in flow may not be detrimental. The electrocardiographic evidence also suggests that prostacyclin may have a direct beneficial action in reducing and/or delaying the effects of acute myocardial ischaemia.

This work was supported by the Scottish Hospitals Research Trust. Prostacyclin was a gift from Dr. J.E. Pike, The Upjohn Co., Kalamazoo, Michigan, U.S.A.

References

DUSTING, G.J., CHAPPLE, D.J., HUGHES, B., MONCADA, S. & VANE, J.R. (1978). Prostacyclin (PGI_2) induces coronary vasodilatation in anaesthetized dogs. *Cardiovasc. Res.*, **12**, 720–730.

MARSHALL, R.J., PARRATT, J.R. & LEDINGHAM, I. McA (1974). Changes in blood flow and oxygen consumption in normal and ischaemic regions of the myocardium following acute coronary artery ligation. *Cardiovasc. Res.* **8**, 204–215.

MARSHALL, R.J. & PARRATT, J.R. (1977). The antidysrhythmic aminosteroid, ORG 6001, reduces ST-segment elevation produced by coronary occlusion in the dog. *Br. J. Pharmac.*, **61**, 315–317.

OGLETREE, M.L., LEFER, A.M., SMITH, J.B. & NICOLAOU, K.C. (1979). Studies on the protective effect of prostacyclin in acute myocardial ischaemia. *Eur. J. Pharmac.*, **56**, 95–103.

The effect of nerve stimulation on release of prostacyclin (PGI_2) from the rat and rabbit mesenteric arteries

E.W. HORTON, E. PIPILI & N.L. POYSER

Department of Pharmacology, University of Edinburgh, 1, George Square, Edinburgh EH8 9JZ, Scotland

Stimulation of adrenergic nerves causes release of prostaglandins, mainly of the E-series, from various tissues (Westfall, 1977). As prostaglandins E_1 and E_2 inhibit noradrenaline release from sympathetic nerves in some tissues (Hedqvist, 1977), it has been suggested that these prostaglandins play a physiological role in the negative feedback control of adrenergic neurotransmission.

Prostacyclin (PGI_2) is the main prostaglandin produced by the blood vessels (Moncada & Vane, 1978). In the present study the release of PGI_2 from rat and rabbit mesenteric arteries during nerve stimulation has been examined.

Male albino rabbits weighing 2–3 kg and male rats weighing 300–350 g were anaesthetized with sodium pentobarbitone (30 mg/kg intravenously and 60 mg/kg intraperitoneally respectively). The abdomen was opened and the superior mesenteric artery was cannulated. The artery was then removed along with its small resistance vessels as described by McGregor (1965). The isolated vessels were flushed with saline and transferred to a thermostatically controlled box. Both rabbit and rat mesenteric vessels were perfused with McEwens' solution (McEwen, 1956), bubbled with 95% O_2, 5% CO_2, at a constant rate of 5 ml/minute. One minute samples of perfusate were collected on a fraction collector. A bipolar platinum electrode was placed around the periarterial nerve plexus. The nerves were stimulated at 10 Hz using supramaximal biphasic rectangular pulses, 1 ms duration, for 15 s

at 5 min intervals. The PGI_2 content of the perfusate samples was estimated in terms of 6-oxo-$PGF_{1\alpha}$ by radioimmunoassay as described by Poyser & Scott (1980). Initially 6-oxo-$PGF_{1\alpha}$ was assayed after ethyl acetate extraction of the perfusate. In later experiments the perfusate was assayed directly; similar results were obtained. The solvent extraction step was therefore omitted. Results were compared by using a Students *t*-test.

PGI_2 was shown to be released at a rate of 3.1 ± 0.5 ng/min ($n = 15$) in the rabbit and at 0.9 ± 0.13 ng/min ($n = 9$) in the rat at rest immediately before stimulation. After each stimulation the amounts of PGI_2 increased and reached within 2 min a maximum of 6.4 ± 0.8 ng/min ($n = 15$) and 1.6 ± 0.2 ng/min ($n = 9$) for the rabbit and rat respectively. This increase in both species was statistically significant ($P < 0.01$).

In experiments using noradrenaline as a stimulus PGI_2 release was also increased.

The preliminary results with noradrenaline would suggest that the increase in PGI_2 release follows the action of noradrenaline at the postsynaptic site. As PGI_2 is a vasodilator substance, the increase in its release may play some role in modifying blood vessel tone.

References

HEDQVIST, P. (1977). Basic mechanisms of prostaglandin action on autonomic neurotransmission. *Ann. Rev. Pharmacol.*, **17**, 259–279.

MCEWEN, L.M. (1956). The effect on the isolated rat heart of vagal stimulation and its modification by cocaine, hexamethonium and ouabain. *J. Physiol.*, **131**, 678–689.

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

MONCADA, S. & VANE, J.R. (1978). Pharmacology and enodgenous roles of prostaglandin endoperoxides, thromboxane A_2 and prostacyclin. *Pharmacol. Rev.*, **30**, 293–331.

POYSER, N.L. & SCOTT, F.M. (1980). Production of prostaglandins and thromboxane *in vitro* by the rat uterus and ovary during the oestrous cycle. *J. Reprod. Fert.*, (in press).

WESTFALL, T.C. (1977). Local regulation of adrenergic neurotransmission. *Physiol. Rev.*, **57**, (4), 659–728.

Antagonism of prostanoid-induced contractions of rat stomach muscle by trimethoquinol or isoxsuprine

SARAH M. LACEY & G.J. SANGER

Department of Surgery, King's College Hospital Medical School, London SE5 8RX

In rat gastric fundus strips the β-adrenoceptor stimulant trimethoquinol (50 ng/ml) greatly reduced longitudinal muscle contraction to various prostanoids (PGD_2, $PGF_{2\alpha}$, epoxymethano analogues of PGH_2, PGI_2, 6-keto-$PGF_{1\alpha}$, 6,15-diketo-$PGF_{1\alpha}$ or thromboxane B_2). However, the reduction of PGE_2 was not significantly greater than the small antagonism of contractions to acetylcholine (ACh) (Bennett & Sanger, 1979; A. Bennett, C. Jarosik, G.J. Sanger & D.E. Wilson, unpublished). Similarly, the β-adrenoceptor stimulant isoxsuprine (0.1–1 μg/ml) preferentially reduced contraction of this tissue to $PGF_{2\alpha}$ or PGE_1 (Coceani & Wolfe, 1966; Wolfe Coceani & Pace-Asciak, 1967). We have now extended these studies, and examined the possible difference in the effects of β-adrenoceptor stimulants on contractions to PGE_1 or PGE_2.

Strips of rat gastric fundus were cut parallel to the longitudinal muscle (Bennett & Sanger, 1979), and cumulative dose-response curves were obtained for ACh, PGE_1, PGE_2, $PGF_{2\alpha}$ or PGI_2. The effects of the β-stimulants were tested using tissues from the same animal, in the presence of vehicle control or 2 μg/ml propranolol which did not greatly affect the concentration of agonist required to produce a 50% maximum contraction (EC_{50}).

Isoxsuprine (0.5 μg/ml) was less potent than trimethoquinol but it also preferentially inhibited $PGF_{2\alpha}$ and PGI_2 (Table 1). However the tendency for a greater effect on PGE_1 compared with that on PGE_2 was not statistically significant.

Thus β-adrenoceptor stimulants inhibit contractions to some prostanoids more than they inhibit contractions to ACh. The results with trimethoquinol indicate that receptors for PGE_2, in contrast to PGE_1, may be resistant to this effect, but the results with the less potent isoxsuprine are less clear.

We thank the MRC for support, the Upjohn Co. Ltd and Wellcome Research for prostanoids, Roche Products Ltd for trimethoquinol and Philips-Duphar for isoxsuprine.

References

BENNETT, A. & SANGER, G.J. (1979). Trimethoquinol selectively antagonises longitudinal muscle contractions of rat isolated gastric fundus to thromboxane B_2 and

Table 1 Increase in EC_{50} required after addition of trimethoquinol (50 ng/ml) or isoxsuprine (0.5 μg/ml) compared with effect on ACh (*P*, Mann-Whitney U-test; Median values are shown with semi-quartile ranges in parentheses). *n* = number of preparations. * $P \leq 0.05$, comparison of tissues with and without propranolol (2 μg/ml) (Wilcoxon matched pairs test)

Agonist	*With trimethoquinol*	*P*	*n*	*With trimethoquinol and propranolol*	*P*	*n*
ACh	3.3 (1.7–4.2)		14	1.6 (1.2–2.9)*		14
PGE_2	3.9 (2.0–7.0)†	>0.1	6	Not tested		
$PGF_{2\alpha}$	10.1 (9.4–23.9)	<0.02	6	3.5 (0.9–6.3)	>0.1	6
PGI_2	169 (143–215)	<0.002	6	16.5 (8.4–25.0)*	<0.002	6
PGE_1	11.5 (7.8–13.6)	<0.02	6	1.9 (1.1–6.9)*	<0.1	6
Agonist	*With isoxsuprine*	*P*	*n*	*With isoxsuprine and propranolol*	*P*	*n*
ACh	2.5 (1.9–3.9)		28	1.8 (1.4–2.3)*		22
PGE_2	3.3 (2.6–4.9)	0.3	6	Not tested		
$PGF_{2\alpha}$	4.3 (3.8–7.8)	0.01	8	2.0 (1.6–2.1)*	0.8	8
PGI_2	6.5 (3.7–8.9)	0.025	6	3.3 (2.0–7.2)	0.03	6
PGE_1	4.7 (2.7–7.1)	0.084	8	2.3 (1.8–2.9)*	0.2	8

† Data from Bennett & Sanger, 1979.

epoxymethano analogues of PGH_2, *Br. J. Pharmac.*, **66**, 450P.

COCEANI, F. & WOLFE, L.S. (1966). On the action of prostaglandin E_1 and prostaglandins from brain on the isolated rat stomach *Can. J. Physiol. Pharmacol.*, **44**, 930–950.

WOLFE, L.S., COCEANI, F. & PACE-ASCIAK, C. (1967). Brain prostaglandins and studies on the action of prostaglandins on the isolated rat stomach. In: *Nobel Sym. II, Prostaglandins, Stockholm*, Ed. Bergstrom, S. & Samuelsson, B., pp. 265–276.

An analysis of the prostanoid receptors mediating contraction of chick isolated ileum

R.A. COLEMAN, I. KENNEDY, G.P. LEVY & CAROL PENNING

Department of Pharmacology, Glaxo Group Research Ltd., Ware Division, Ware, Herts

We have previously suggested that prostanoid-induced contraction of guinea-pig ileum and fundus is mediated by a single type of receptor characterised by the order of agonist potency $PGE_2 > PGE_1 > PGF_{2\alpha}$, and which is selectively blocked by SC-19220 (Coleman, Kennedy & Levy, 1980). In this communication we report an analysis of the receptors mediating contraction of another preparation which is sensitive to E-series prostaglandins, the chick isolated ileum.

Segments of ileum about 1 cm long were removed from 5–20 day old chicks (RIR × LSL strain) and suspended in modified Krebs solution containing antagonists (Apperley, Coleman, Kennedy & Levy, 1979) at 37°C and gassed with 95% O_2:5% CO_2. A resting tension of 1 g was applied. Agonist concentration-effect curves were constructed cumulatively, PGE_1 being used as a standard agonist in each experiment. The preparation was contracted by PGE_1 (EC_{50} 1.7 ng/ml, 95% confidence limits 0.8–3.6 ng/ml, $n = 28$). PGE_2 was 4 (1.9–9.4, $n = 11$) times and $PGF_{2\alpha}$ 237 (95–594, $n = 11$) times less potent than PGE_1. At a concentration of 3×10^{-4} mol/l (contact time 45 min), SC-19220 had no effect on responses of the ileum to PGE_1, PGE_2 or $PGF_{2\alpha}$.

The order of agonist potency ($PGE_1 > PGE_2 > PGF_{2\alpha}$) and the lack of effect of SC-19220 suggest that contraction of chick ileum is not mediated by the same type of prostanoid receptor as is contraction of guinea-pig ileum and fundus (Coleman *et al*, 1980). Accordingly, it is likely that there are at least two types of receptor mediating

smooth muscle contraction at which E-series prostaglandins are the most potent agonists. In this context it is interesting to note that there is at least one other smooth muscle preparation, human stomach longitudinal muscle, where PGE_2 is a more potent contractile agonist then $PGF_{2\alpha}$ (Adaikan & Karim, 1976), and where SC-19220 is ineffective as an antagonist (Bennet & Posner, 1971).

PGE_1 is more potent than PGE_2 in contracting chick ileum (this study) and as an inhibitor of aggregation of human platelets (MacIntyre & Gordon, 1975). However, PGI_2 is more potent than PGE_1 on platelets (Whittle, Moncada & Vane, 1978), whereas PGI_2 is 36 (14–96, $n = 6$) times less potent than PGE_1 on chick ileum. It is unlikely therefore that the prostanoid receptor in chick ileum is the same as that in human platelets. However, since a similar order of agonist potency to that on chick ileum has been described in a number of systems in which prostaglandins sensitize peripheral nociceptors (Tyers & Haywood, 1980), the latter effect could be mediated through similar prostanoid receptors to those mediating contraction of chick ileum.

References

ADAIKAN, P.G. & KARIM, S.M.M. (1976). Effects of PGA and PGB compounds on gastrointestinal tract smooth muscle from man and laboratory animals. *Prostaglandins*, **11**, 15–22.

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

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

COLEMAN, R.A., KENNEDY, I. & LEVY, G.P. (1980). SC-19220, a selective prostanoid antagonist. *Br. J. Pharmac.* In press.

MACINTYRE, D.E. & GORDON, J.L. (1975). Calcium-dependent stimulation of platelet aggregation by PGE_2. *Nature*, **258**, 337–339.

TYERS, M.B. & HAYWOOD, HAZEL (1980). Effects of prostaglandins on peripheral nociceptors in acute inflammation. *Agents and Actions*, in press.

WHITTLE, B.J.R., MONCADA, S. & VANE, J.R. (1978). Comparison of the effects of prostacyclin (PGI_2), prostaglandin E_1 and D_2 on platelet aggregation in different species. *Prostaglandins*, **16**, 373–388.

Effect of prostaglandin synthetase inhibitors on indirectly evoked contractions of whole preparation and longitudinal muscle strips of guinea-pig ileum

YETUNDE O.O. SOKUNBI
(Introduced by D.T. OKPAKO)

Department of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria

Prostaglandin synthetase inhibitors (PGSI) block contractions evoked by transmural stimulation (TMS) and nicotine but not acetylcholine in the guinea-pig isolated ileum (Ehrenpreis, Greenberg & Belman (1973); Bennett, Eley & Stockley (1975). The inhibited responses can be restored by the addition of low doses of PGE_2. It has therefore been suggested that a prostaglandin may play a facilitatory role in cholinergic transmission in the guinea-pig ileum (Sokunbi, 1979). The interpretation of the action of PGSI however is complicated since the large doses required can have effects other than the inhibition of prostaglandin synthesis.

The need for high doses of PGSI has been attributed to the presence of diffusion barriers or differences in the sensitivities of neuronal or extraneuronal synthetases to PGSI (Bennett *et al.*, 1975). Indeed, indomethacin is more potent in blocking PG synthetase in homogenates than in slices of seminal vesicle (Raz, Stern & Kenig-Wakshal, 1973).

The effects of PGSI on indirectly evoked contractions of the whole ileum preparation (WIP) and those of longitudinal muscle strips (LMS) in which Auberbachs plexus is exposed are now reported. LMS were prepared as described by Paton & Zar (1968) and in Krebs solution containing choline chloride (20 μM) at 37°C. The solution was gassed with 5% CO_2 in oxygen. Isometric contractions were recorded on Devices MX212 recorder from a baseline tension of 0.5 g.

Indomethacin and ketoprofen (40 μg/ml each) reduced contractions evoked by TMS (0.1 Hz, 2 ms, supramaximal voltage) by 17% and 12% ($n = 6$) respectively in WIP; whereas in the LMS 1μg ml^{-1} of both drugs reduced contractions by 28% and 34% ($n = 6$) respectively. Sodium meclofenamate (0.5 μg/ml) inhibited TMS evoked contractions by 35% in LMS whereas 5 μg/ml had no effect on WIP ($n = 6$). Indomethacin (10 μg/ml) and ketoprofen (10 μg/ml) and sodium meclofenemate (5 μg/ml) caused a significant reduction in nicotine-evoked contractions in WIP. In the LMS however, $1/10^{th}$ of these contractions caused a more marked reduction in nicotine contractions. As in the whole ileum, contractions evoked by acetylcholine on the LMS were unaffected by, PGSI. The inhibitions were all reversed by 1–2.5 ng/ml of PGE_2.

The results suggest that the action of PGSI in isolated guinea-pig ileum is influenced by diffusion barriers. The present finding that PGSI in low doses caused PGE_2-reversible block of indirectly evoked contractions of the guinea-pig ileum strengthens the

proposition that a prostaglandin facilitates cholinergic transmission in this preparation.

References

BENNETT, A., ELEY, K.G. & STOCKLEY, H.L. (1975). "Modulation by prostaglandins of contractions in guinea-pig ileum". *Prostaglandins*, **9**, No. 3 377–384.

EHRENPREIS, S., GREENBERG, J. & BELMAN, S. (1973). "Prostaglandins reverse inhibition of electrically induced contractions of guinea-pig ileum by morphine, indomethain and acetylsalicylic acid. *Nature (New Biology)*, **245**, 280–282.

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

RAZ, A., STERN, H. & KENIG-WAKSHAL, R. (1973). Indomethacin and aspirin inhibition of prostaglandin E_2 synthesis by sheep seminal vesicle microsome powder. *Prostaglandins*, **3**, 337–52.

SOKUNBI, Y.O.O. (1979). Does a prostaglandin modulate cholinergic transmission in the guinea-pig ileum 3. *Br. J. Pharmac.*, **6**, 464P.

Reversal of α-adrenoceptor blockade by PGE_2 in rat isolated mesenteric artery

A.S.O. ADEAGBO (Introduced by D.T. OKPAKO)

Department of Pharmacology & Therapeutics, University of Ibadan, Ibadan, Nigeria

In the isolated mesenteric artery preparation, aspirin and indomethacin reduce the vasoconstrictor action of noradrenaline (NA) (Couper & McLennan, 1978). The inhibitory effect of the PG synthetase inhibitor is reversed by low concentrations of PGE_2 implying that the action of NA is mediated by a prostaglandin. We report here that competitive block of NA vasoconstriction caused by phentolamine, tolazoline, and yohimbine in the isolated mesenteric artery is also reversed by PGE_2.

The isolated mesenteric artery preparation was set up as described by McGregor, (1965) and perfused at 4 ml/min with Krebs solution at 37°C. The solution was gassed with 5% CO_2 in oxygen. Changes in perfusion pressure were measured by Bell & Howell pressure transducer on a Devices M19 recorder.

Phentolamine (3.2×10^{-8} M), tolazoline (4×10^{-5} M), and yohimbine (12.8×10^{-7}M) caused competitive block of NA with dose ratios of 13.9 ± 1.0, 22.0 ± 1.0 and 26.6 ± 0.9 respectively ($n = 8$ in each case). The dose ratios were reduced to 2.5 ± 0.1, 5.9 ± 0.4 and 1.7 ± 0.1 in the presence of PGE_2 (2.8×10^{-8} M). Non-competitive block of NA caused by phenoxybenzamine and prazosin were not reversed by PGE_2. Block of NA by competitive antagonists in Ca^{2+}-free Krebs was also reversed by PGE_2.

The reversal of block was not due to alteration of α-adrenoceptor in such a way as to reduce its affinity for the antagonist since pA_2 values (Arunlakshana & Schild, 1959) in the presence of PGE_2 were even higher than those obtained in Krebs solution. The mechanism of reversal of blockade seems also to be different from that by which PGE_2 potentiates NA since potentiation is absent in Ca^{2+}-free Krebs (Adeagbo & Okpako, 1980). These results demonstrates that the antagonism of NA by competitive α-adrenoceptor blockers which are not known to block PG synthesis or receptor can be reversed by PGE_2.

References

ADEAGBO, A.S.O. & OKPAKO, D.T. (1980). Mechanism of Noradrenaline potentiation by PGE_2 in rat mesenteric artery. *Br. J. Pharmacol.*, (1980)—In press.

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

COUPER, K.M. & MCLENNAN (1978) The influence of prostaglandins on Noardrenaline-induced vasoconstriction in isolated perfused mesenteric blood vessels of the rat. *Br. J. Pharmac.*, **62**, (1) 51–59.

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

Spontaneous and evoked release of [^{3}H]-spermine from rat cerebral cortex slices

R.J. HARMAN & G.G. SHAW

Department of Pharmacy, University of Nottingham, Nottingham NG7 2RD

Investigations concerned with elaborating the functions of polyamines in the central nervous system have recently been reviewed (Shaw, 1979) and include some findings which are consistent with a transmitter or modulator role for spermine.

In preliminary experiments using 0.1 mm slices of rat cerebral cortex, a two component uptake system for spermine has been identified. Kinetic analysis (Neal, 1972) indicated K_m values of 580 and 5.5 nM.

This communication describes an investigation of

the release of [^{3}H]-spermine from 30 mg aliquots of 0.1 mm slices which had been pre-incubated (20 min, 37°C) with 5.65 nM [^{3}H]-spermine when some 85% of the total uptake occurs via the higher affinity system. After pre-incubation, the slices were filtered on a 2.5 cm Millipore membrane (pore size 0.8 μm), transferred to a Swinnex filter holder and perfused at 0.5 ml/min with Kreb's solution. Fractions of perfusate were collected at 2 min intervals. The [^{3}H]-spermine content of 0.5 ml samples of perfusate, and of the residual tissue which was solubilised together with the membrane in Protosol, was measured by liquid scintillation spectrometry. Spontaneous release, expressed as the fractional rate coefficient (Hopkin & Neal, 1971) was biphasic and consisted of an initial rapid efflux (K 0.274 min^{-1}) and a slower component (K 0.023 min^{-1}). In experiments in which release was evoked by depolarising stimuli, the tissue was perfused for 20 min before fractions were collected to avoid the initial rapid efflux. Stimuli (50 mM potassium, 100 μM ouabain or 10 μg ml^{-1} veratrine) were applied for 4 min beginning at 26 min and 40 minutes.

The introduction of potassium doubled the rate of efflux and released 7.9 ± 0.9% (mean ± s.e. mean, $n = 7$) of total tissue radioactivity. In calcium-free conditions, release evoked by potassium was reduced by 64%. The second application of potassium in the presence of a normal concentration of calcium produced a response which was only 20% as large as the first. Clearly, the spermine pool whose release is calcium dependent is exhausted by the first application of potassium.

Release evoked by ouabain was independent of calcium. Ouabain produced a 2.5 to 3 fold increase in efflux rate, representing the release of 8.2 ± 1.0% ($n = 8$) of the total tissue radioactivity in the presence of calcium, and 8.6 ± 0.2% ($n = 8$) in its absence. The second response to ouabain was greatly reduced both with normal and calcium free media.

Veratrine produced a small, but significant, release of spermine in normal medium amounting to some 5.3% of total tissue radioactivity. However, in calcium free medium, a slow and sustained increase in efflux occurred.

These findings provide encouragement for further investigation of a possible neurohumoral role for spermine.

R.J.H. is supported by an S.R.C. research studentship.

References

HOPKIN, J. & NEAL, M.J. (1971). Effect of electrical stimulation and high K^+ concentrations on the efflux of ^{14}C-glycine from slices of spinal cord. *Br. J. Pharmac.*, **42**, 215–223.

NEAL, J.L. (1972). Analysis of Michaelis Menten kinetics for two independent saturable membrane transport functions. *J. Theor. Biol.*, **35**, 113–118.

SHAW, G.G. (1979). The polyamines in the central nervous system. *Biochem. Pharmac.*, **28**, 1–6.

The effects of drugs on cerebral cortex ATP levels in normal and hypoxic rats

P.M. PACIOREK & M.G. WYLLIE

Department of Pharmacology, Wyeth Institute of Medical Research, Huntercombe Lane South, Taplow, Maidenhead, Berkshire

The excitability of neurones is controlled by a variety of processes. One of the most important enzymes controlling CNS excitability is sodium, potassium-activated, magnesium-dependent adenosine triphosphatase (Na^+, K^+-ATPase, EC.3.6.1.3) which is involved not only in the maintenance of the resting membrane potential but also in neurotransmitter release (Paton, Vizi & Aboo-Zar, 1971; Hokin & Dahl, 1972; Gilbert, Wyllie & Davison, 1975). Inhibition of Na^+, K^+-ATPase by the classical inhibitor ouabain or pentylenetetrazol can result in pronounced behavioural effects (Bignami & Palladini, 1966; De Robertis, Rodriguez De Lores Arnaiz & Alberici, 1969).

The activity of Na^+, K^+-ATPase is regulated by the amount of substrate (ATP) present. Normally because of adequate synthesis, the levels of ATP are rarely limiting. However, under certain stress or age-dependent conditions cellular synthesis may be impaired. A variety of compounds including hydergine, pemoline, meclofenoxate, and naftidrofuryl have been suggested as general CNS activators and we have therefore studied their effects along with that of the related indoramin on high-energy phosphate levels and utilisation.

Hypoxic conditions (PO_2, 21 mmHg) resulted in a significant decrease in rat cerebral cortex ATP levels

Table 1 The effects of drugs on rat cerebral cortex ATP levels in normal and hypoxic rats

Treatment	Dose ($mg.kg^{-1}$ p.o.)	ATP levels* $\mu mol\ ATP.g\ wet\ weight\ cortex^{-1}$ Normal	Hypoxic**
HPMC (Control)		2.64 ± 0.07	1.58 ± 0.02
Hydergine	3	2.58 ± 0.07	2.36 ± 0.03†
Indoramin	3	2.62 ± 0.10	2.39 ± 0.03†
Naftidrofuryl	10	2.60 ± 0.05	2.36 ± 0.07†
Pemoline	10	2.59 ± 0.13	1.54 ± 0.03
Meclofenoxate	10	2.54 ± 0.04	1.53 ± 0.03

* All values are the mean ± s.e. mean of 4–6 experiments. ATP was measured fluorimetrically or by luciferin-luciferase.

** Animals were gassed in a sealed system with a special gas mixture (PO_2, 21 mmHg), 6 l.min prior to sacrifice. In all cases brains were rapidly removed and frozen on dry-ice.

† Statistically significant protection against hypoxia $P < 0.001$.

(Table 1). Although neither pretreatment with hydergine (3 mg/kg); indoramin (3 mg/kg) nor naftidrofuryl (10 mg/kg) altered brain ATP levels in the normal animal, they each protected against the fall due to hypoxia. On the other hand, pemoline (10 mg/kg) and meclofenoxate (10 mg/kg) were ineffective in both normal and hypoxic animals.

A similar drug-reversal of ATP depletion was seen *in vitro* using the method of Keen & White (1970) to study synaptosomal swelling. Hypoxia (nitrogen gassing), administration of either metabolic inhibitors or the Na^+, K^+-ATPase inhibitor ouabain produced synaptosomal swelling. The effects of hypoxia could be partially prevented by hydergine (0.1 mm), indoramin (0.1 mM) or naftidrofuryl (0.1 mM), but not by pemoline (5.7 μM) or meclofenoxate (0.1 mM).

The relevance of these findings to general CNS excitability was seen on studies on rat EEG. The metabolic inhibitor KCN (0.4 mg kg^{-1} min^{-1}) produced an abnormal EEG, the onset of which was prolonged by pretreatment with indoramin (6 mg kg) but not meclofenoxate (100 mg/kg).

On this basis, the CNS activators studied fall into two categories: drugs which prevent the hypoxia-mediated reduction in CNS ATP levels and those which do not. The latter group (pemoline and meclofenoxate) may act by a direct effect on Na^+, K^+-ATPase (Gilbert, Allen, Townsend and Wyllie, 1978). A more indirect action on Na^+, K^+-ATPase involving a cell-swelling model is proposed to account for the actions of hydergine, indoramin and naftidrofuryl, where a reduction in swelling of the capillary endothelium facilitates oxygen diffusion.

References

BIGNAMI, A. & PALLADINI, G. (1966). Experimentally produced cerebral status spongiosus and continuous pseudorhythmic electro encephalographic discharges with membrane ATPase inhibitor in rat. *Nature, Lond.*, **209**, 413–414.

DE ROBERTIS, E., RODRIGUEZ DE LORES ARNAIZ, G. & ABERICI, M. (1969). Ultrastructural Neurochemistry. In, Basic Mechanisms of the Epilepsies. ed. *Jasper, H.H., et al*, pp. 137–158. Churchill, London.

GILBERT, J.G., ALLEN, J.M., TOWENSEND, B.G. & WYLLIE, M.G. (1978). Drugs affecting the central nervous system: Effects of pemoline and tri-cyclic antidepressants on nerve terminal adenosine-triphosphatase activities and neurotransmitter release. *Neuropharmacology*, **17**, 419–421.

GILBERT, J.C., DAVISON, D.V. & WYLLIE, M.G. (1975). Nerve terminal ATPase as a possible trigger for neurotransmitter release. *Nature, Lond.*, **225**, 237–238.

HOKIN, L.E. & DAHL, J.L. (1972). The sodium-potassium adenosine triphosphatase. In *Metabolic Pathways, Metabolic Transport, ed. Hokin, L.E.*, **Vol. VI.** pp. 267–315. Academic Press, New York.

KEEN, P. & WHITE, T.D. (1970). Light-scattering technique for the study of the permeability of rat brain synaptosomes *in vitro*. *J. Neurochem.*, **17**, 565–571.

PATON, W.D.M., VIZI, E.S. & ABOO-ZAR, M. (1971). The mechanism of acetylcholine release from parasympathetic nerves. *J. Physiol., Lond.*, **215**, 819–848.

Stimulation of rat cerebellar cyclic GMP concentrations *in vitro* by glutamate-receptor agonists

G.A. FOSTER & P.J. ROBERTS

School of Biochemical and Physiological Sciences, Department of Physiology and Pharmacology, University of Southampton, Southampton, SO9 3TU

The actions of certain neurotransmitters in the mammalian CNS may be associated with the formation of cyclic GMP. In the cerebellum, glutamate is likely to be the excitatory transmitter released from parallel fibre terminals, and in this tissue it has been shown to stimulate the formation of cyclic GMP, both in slices (Ferendelli, Chang & Kinscherf, 1974; Schmidt, Ryan & Molloy, 1976; Schmidt, Thornberry & Molloy, 1977; Garthwaite & Balázs, 1978) and *in vivo*, after intra-cerebroventrivular injection (Briley, Kouyoumdjian, Haidamous & Gonnard, 1979).

In this study, we have investigated the pharmacological specificity of the cGMP response to exogenous glutamate in the cerebellum. Cerebellar slices (0.5 mm × 0.5 mm) from 8-day old female rats, were preincubated for 90 min at 37°C in Krebs-bicarbonate medium under 95% O_2: 5% CO_2 at a tissue concentration of approximately 20 mg/ml, followed by incubation for a further 5 min in the presence of glutamate or other agonist substance. Potential antagonists were tested by their inclusion 15 min prior to addition of the agonist.

Incubations were terminated by aspiration of the Krebs medium, and its replacement with 0.05 M Tris-HCl (pH 7.5) containing 4 mM EDTA, followed by heating at 100°C for 10 min. After homogenisation and centrifugation, 100 µl aliquots of the supernatant were assayed for cGMP, using commercial kits.

L-glutamate produced a dose-related increase in the concentration of cGMP in cerebellar slices, with the half-maximal response occurring at approximately 1 mM (Figure 1).

A number of related amino acids were also able to raise cyclic GMP levels, with a rank order of potency similar to that for excitation of mammalian spinal neurons i.e. N-methyl-D-aspartate (NMDA) > 4-fluoroglutamate > DL-homocysteate = DL-ibotenate > L-glutamate = L-aspartate = kainate > dihydrokainate. Kainate itself however, was unable to evoke the same maximal response as L-glutamate.

L-glutamate diethylester inhibited the stimulation by glutamate, aspartate and NMDA, while D-α-aminoadipate, and DL-α-aminosuberate, were inactive.

It is concluded that the receptor mediating the stimulation of cyclic GMP levels by glutamate, closely resembles the glutamate receptor, defined electrophysiologically.

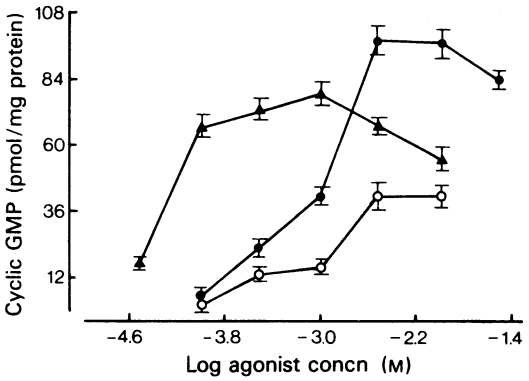


Figure 1 Effect of L-glutamate (●), N-methyl-D-aspartate (▲) and kainate (○) on cerebellar slice cyclic GMP concentrations. Results are means ± s.e. mean of at least 3 experiments.

G.A.F. is an SRC Research Student.
We thank the S.K. & F. Foundation for a Research Grant, and J.C. Watkins (Bristol) for the gift of various compounds.

References

BRILEY, P.A. KOUYOUMDJIAN, J.C., HAIDAMOUS, M. & GONNARD, P. (1979) Effect of L-glutamate and kainate on rat cerebellar cGMP *in vivo*. *Eur. J. Pharmacol.*, **54**, 181–184.

FERENDELLI, J.A., CHANG, M.-M. & KINSCHERF, D.A. (1974) Elevation of cyclic GMP levels in central nervous system by excitatory and inhibitory amino acids. *J. Neurochem.*, **22**, 535–540.

GARTHWAITE, J. & BALÀZS, R. (1978) Supersensitivity to the cyclic GMP response to glutamate during cerebellar maturation. *Nature*, **275**, 328–329.

SCHMIDT, M.J., RYAN, J.J. & MOLLOY, B.B. (1976). Effects of kainic acid, a cyclic analogue of glutamic acid, on cyclic nucleotide accumulation in slices of rat cerebellum. *Brain Res.*, **112**, 113–126.

SCHMIDT, M.J., THORNBERRY, J.F. & MOLLOY, B.B. (1977). Effects of kainate and other glutamate analogues on cyclic nucleotide accumulation in slices of rat cerebellum. *Brain Res.*, **121**, 182–189.

Effect of anti-anxiety drugs, amphetamine and chloral hydrate on social interaction and locomotion in rats

S.B. KIDD & C.R. GARDNER

Roussel Laboratories, Kingfisher Drive, Covingham, Swindon, Wiltshire

Social interaction (SI) is reduced in a well-lit novel environment and reversal of this has been claimed to be an action of anti-anxiety drugs (File & Hyde, 1978). We have investigated the specificity of this response by comparing the effects of anti-anxiety agents with those of (+)-amphetamine sulphate and chloral hydrate.

Male Wistar rats (200–300 g) were housed singly for 5–6 days prior to testing. Pairs of weight matched rats were observed for 6 min in a wooden box (46 × 46 × 38 cm). The time spent in active SI was recorded on a stopwatch (File & Hyde, 1978). Walks greater than one body length and rears were also scored. Full submissive postures were also recorded as an independent measure of aggression. SI was observed in a sound attenuation chamber under four conditions: after familiarisation with the box, under high (HF) or low (LF) lighting; and without familiarisation under high (HU) or low (LU) lighting.

Both high light and novelty reduced SI (LF: 176 ± 7s mean ± s.e. mean, HF: 137 ± 9s, LU: 133 ± 9s, HU: 81 ± 4s; LF-HU, $P < 0.001$ Student's t test), particularly aggression (LF: 19 ± 1, HF: 5 ± 1, LU: 7 ± 1, HU: 1 ± 0.5; LF − HU, $P < 0.001$). Walks showed a smaller decrease across the four conditions (LF: 102 ± 8, HF: 96 ± 7, LU: 86 ± 6, HU: 78 ± 3; LF-HU, $P < 0.005$) whilst rears were reduced by high light but unaltered by familiarisation (LU: 61 ± 4, HU: 46 ± 2; $P < 0.005$ and LF: 62 ± 4, HF: 39 ± 2; $P < 0.001$).

Housing and familiarising animals in pairs (LF paired) also reduced SI (83 ± 7s) walks (63 ± 4) and aggression (2 ± 1) without affecting rears (64 ± 6). Animals in the HU condition showed less SI (65 ± 3) outside the sound chamber under quiet laboratory conditions.

Chlordiazepoxide (5 mg/kg i.p.) increases SI in these conditions (+26s, $P < 0.02$). Similar small increases in SI were observed with chronic (5 daily doses) chlordiazepoxide 5 mg/kg i.p. (+35s, $P < 0.02$) and after intraperitoneal phenobarbitone 7.5 mg/kg (+40s, $P < 0.05$) and 15 mg/kg (+53s, $P < 0.005$), ethanol 0.4 g/kg (+29s, $P < 0.025$) and 1.6 g/kg (+27s, $P < 0.05$), chloral hydrate 50 mg/kg (+33s, $P < 0.005$) and amphetamine 1.5 mg/kg (+36s, $P < 0.005$) and 3.0 mg/kg (+26s, $P < 0.05$). Meprobamate (50–200 mg/kg) had no effect.

Walks were increased with acute and chronic chlordiazepoxide (5 mg/kg), phenobarbitone (15 mg/kg), chloral hydrate (50 mg/kg), amphetamine (3 mg/kg) and meprobamate (100 mg/kg). Rearing was little affected except for increases with amphetamine (1.5 and 3.0 mg/kg) and chloral hydrate (50 mg/kg). There were no drug induced increases in aggression.

Chlordiazepoxide (2.5–10 mg/kg) did not increase SI under the HU condition in the sound chamber. Other factors besides anxiety may reduce SI in these test conditions, possibly a shift from territorial behaviour in familiarised animals to exploration in unfamiliarised animals.

Anxiolytic-induced SI increases (HU, outside the sound chamber) were small and similar effects were observed with amphetamine and chloral hydrate. SI may be influenced by drug changes in behavioural activity.

Reference

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

The effects of capsaicin on the isolated spinal cord of the immature rat

B. AULT (introduced by R.H. EVANS)

Department of Pharmacology, Medical School, University of Bristol, Bristol BS8 1TD

Capsaicin produces a long lasting desensitisation to chemogenic pain associated with loss of sensory neurones (Jancsó, Kiraly & Jancsó-Gábor, 1977). This effect has been correlated with the release of substance P from the spinal cord (Jessel, Iversen & Cuello, 1978), which has been suggested as the transmitter mediating capsaicin-induced depolarization of motoneurones (Theriault, Otsuka & Jessel, 1979).

The present investigation is concerned with the effect of capsaicin on the isolated hemisected spinal cord of the immature rat (Otsuka & Konishi, 1974) in which capsaicin (1 μM) produced depolarization measured in both dorsal and ventral roots. The

capsaicin-induced depolarization of ventral roots was almost abolished by tetrodotoxin (0.1 μM) which suggests that it was produced indirectly. Baclofen (10 μM), a selective depressant of neutransmission mediated through acidic amino acid receptors (Ault & Evans, 1978), and α,ε-diaminopimelic acid (3 mM), a specific antagonist of excitant amino acids (Evans & Watkins, 1978), also reduced capsaicin induced depolarizations in the ventral roots. It is therefore possible that these depolarizations are mediated by acidic amino acids released from interneurones onto motoneurones.

References

AULT, B. & EVANS, R.H. (1978). Central depressant action of baclofen. *J. Physiol. (Lond.)*, **284**, 131P.

EVANS, R.H. & WATKINS, J.C. (1978). Specific antagonism of excitant amino acids in the isolated cord of the neonatal rat. *Eur. J. Pharmacol.*, **50**, 123–129.

JANCSÓ, G., KIRALY, E. & JANCSÓ-GÁBOR, A. (1977). Pharmacologically induced selective degeneration of chemosensitive primary sensory neurones. *Nature (Lond.)*, **270**, 741–743.

JESSEL, T., IVERSEN, L.L. & CUELLO, A.C. (1978). Capsaicin-induced depletion of substance P from primary sensory neurones. *Brain Res.*, **152**, 183–188.

OTSUKA, M. & KONISHI, S. (1974). Electrophysiology of mammalian spinal cord *in vitro*. *Nature (Lond.)*, **252**, 733–734.

THERIAULT, E., OTSUKA, M. & JESSEL, T.M. (1979). Capsaicin-evoked release of substance P from primary sensory neurones. *Brain Res.*, **170**, 209–213.

The antinociceptive effects of single doses of capsaicin in the rodent

A.G. HAYES, M. SKINGLE & M.B. TYERS

Pharmacology Dept., Glaxo Group Research Ltd., Ware, Herts

High doses of capsaicin given repeatedly to rats for 5 days depletes substance P from primary afferent neurones (Jessell, Iversen & Cuello, 1978; Hayes & Tyers, 1980). Local or systemic injection causes an initial stimulation of peripheral nociceptors, followed by a long-lasting insensitivity to chemical noxia (Janscó, 1966). The present study reports the antinociceptive activity of single doses of capsaicin against nociceptive chemical, thermal and pressure stimuli. Capsaicin was suspended in 5% gum acacia. All testing was carried out blind.

In antinociceptive tests in the mouse (male, AHM/ICI, 18–23 g), capsaicin, given subcutaneously (s.c.) under anaesthesia inhibited both acetylcholine and phenylquinone-induced writhing; the ED_{50} (95% confidence limits) values were 1.3 (0.6–2.1) and 1.4 (0.8–2.6) mg/kg respectively. However, in the hot plate (55°C) test, capsaicin (1–20 mg/kg s.c.), had no effect on reaction latencies while the antinociceptive activity (ED_{50}) of morphine in the same test, was 3.6 (1.2–9.7) mg/kg s.c.

In the rat (AH hooded, male, 250–350 g) the effects of capsaicin on chemical nociception were investigated using the method of Dubuisson & Dennis (1977). In this test the nociceptive responses induced by an injection of formalin (5%) into the plantar surface of a forepaw were scored during an observation period up to 1 h after injection. The nociceptive response induced by formalin was effectively reduced by capsaicin (0.3–10 mg/kg s.c.). The antinociceptive effect was dose-related and long-lasting (> 60 min).

The effects of capsaicin on nociceptive pressure thresholds in the hind paws of weanling rats (40–80 g) were determined using an 'analgesymeter' (Ugo Basile) as described by Hayes & Tyers (1980). The antinociceptive activity of capsaicin against pressure nociception was dose-related with an ED_{50} (95% confidence limits) value of 0.37 (0.03–1.04) mg/kg s.c. Peak effect was obtained after 20 min, and the effect lasted > 4 hours. However, capsaicin (30 mg/kg s.c.) was ineffective against heat nociceptive stimuli in the tail immersion (50°C) and hot plate (55°C) tests in the rat. Capsaicin was also effective against pressure nociception when given orally, with ED_{50} (95% confidence limits) value of 62.2 (28–122) mg/kg orally. However, capsaicin 300 mg/kg orally, was ineffective against noxious heat stimuli. Capsaicin (12.5–100 μg) was also administered to rats (male AH hooded, 250 g) by the intrathecal route under anaesthesia, according to the method of Yaksh & Rudy (1976), the drug being dissolved in 50% DMSO/50% saline. Capsaicin, (25–100 μg) caused dose-related elevation of nociceptive thresholds (non-parametric statistical test—Shirley, 1977) in the paw pressure test 1 h and 24 h after dosing; no significant effects were obtained 4 days after dosing. These doses of capsaicin had no significant dose-related effects in the tail immersion or hot plate tests at any time.

These results reinforce the findings of Hayes & Tyers (1980) in which adult rats treated repeatedly with capsaicin showed increased nociceptive pressure and chemical thresholds while nociceptive heat thresholds were unchanged.

References

DUBUISSON, D. & DENNIS, S.G. (1977) The formalin test. *Pain*, **4**, 161–174.

HAYES, A.G. & TYERS, M.B. (1980). Capsaicin depletes substance P from dorsal norn and skin and discriminates heat from chemical and pressure nociceptive stimuli in the conscious rat. *J. Physiol.*, (in press).

HAYES, A.G. & TYERS, M.B. (1980). Effect of capsaicin on nociceptive heat, pressure and chemical thresholds and on substance P levels in the rat. *Brain Research*, (in press).

JANSCÓ, N. (1966). Desensitization with capsaicin and related acylamides as a tool for studying the function of pain receptors.. In: *Pharmacology of Pain. Proc. 3rd Int. Pharmacol. meeting* (publisher Pergamon Press).

JESSELL, T.M., IVERSEN, L.L. & CUELLO, A.C. (1978). Capsaicin induced depletion of substance P from 1° sensory neurones. *Brain Res.*, **152**, 183–188.

SHIRLEY, E. (1977). A non-parametric equivalent of Williams' test for contrasting increasing dose levels of a treatment. *Biometrics*, **33**, 386–389.

YAKSH, T.L. & RUDY, T.A. (1976). Analgesia mediated by a direct spinal action of narcotics. *Science*, **192**, 1357–1358.

Dissimilar effects of cycloheximide on the febrile responses to the intravenous and intracerebroventricular administration of various pyrogens

A.S. MILTON & VINITI K. SAWHNEY

Department of Pharmacology, University of Aberdeen, Marischal College, Aberdeen AB9 1AS, Scotland

It was reported by Seigert, Philipp-Dormston, Radsak & Menzel in 1976 that in the rabbit the protein synthesis inhibitor cycloheximide prevented the induction of the febrile response to intravenously administered exogenous and endogenous pyrogens. To try and obtain information on the possible site of action of cycloheximide the present study was undertaken to examine the effects of cycloheximide (5 mg/kg i.v.) pretreatment, using male and female Dutch rabbits, on the pyrogen responses to intravenous bacterial pyrogen (Shigella dysenteriae 1 μg/kg), or rabbit endogenous pyrogen (4.2×10^8 cell equivalents) and to intracerebroventricular bacterial pyrogen (1 μg/kg), arachidonic acid (100 μg) and prostaglandin E_2 (PGE_2 1 μg). The cycloheximide was injected 90 min before the administration of the pyrogenic agent. With the exception of arachidonic acid all the responses were measured with the animals exposed to three different ambient temperatures, 5°, 20° and 30°C. In the case of arachidonic acid only 20°C was studied.

Cycloheximide blocked the febrile response to intravenous Shigella dysenteriae and to intravenous endogenous pyrogen at all three ambient temperatures. These results differ from those of Stitt who in 1980 reported that the febrile response to an intravenous dose of *E. coli* (5 μg/kg) administered to a rabbit at an ambient temperature of 30°C was not affected by pretreatment with cycloheximide (5 μg/kg). In contrast, the responses to central injections of either bacterial pyrogen or PGE_2 after cycloheximide pretreatment depended upon the ambient temperature. At 5°C cycloheximide attenuated the febrile response to both intracerebroventricular pyrogen and the PGE_2. However, at the two higher temperatures, no such effect was apparent although the febrile responses to intravenous pyrogen were still abolished. In fact, the response to PGE_2 (1 μg) at 20°C appeared to be slightly potentiated. It was found that at 20°C arachidonic acid fever was not affected by cycloheximide pretreatment and this is in agreement with results already reported by Cranston, Dawson, Hellon & Townsend (1978). The immediate effect of cycloheximide (5 mg/kg) on the thermoregulatory response of the animals was a fall in rectal temperature which was significantly greater at 5°C than at 20° or 30°C. This initial hypothermic effect was usually maximal at 45–90 min after injection and in most experiments was followed by a gradual rise in body temperature, the magnitude of which varied greatly.

It appears that cycloheximide may be acting to block at more than one step along the fever pathway and that the peripheral and central inductions of fever differ. Because of the general body requirements for protein synthesis and because of the non-specific nature of cycloheximide it is difficult to decide whether the antipyretic action of cycloheximide demonstrates a role for protein synthesis in fever.

The prostaglandin E_2 was kindly supplied by the Upjohn Company, U.S.A.

References

CRANSTON, W.I., DAWSON, N.J., HELLON, R.F. & TOWNSEND, Y. (1978). Contrasting actions of cycloheximide

on fever caused by arachidonic acid and by pyrogen. *J. Physiol. (Lond.)*., **185**, 35P.

SEIGERT, R., PHILIPP-DORMSTON, W.K., RAIDSAK, K. & MENZEL, H. (1976). Mechanism of fever induction in rabbits. *Infection and Immunity*, **14**, 1130–1137.

STITT, J.T. (1980). The effects of cycloheximide on temperature regulation and fever production in the rabbit in Thermoregulatory Mechanisms and their Therpaeutic Implications, eds: B. Cox, P. Lomax, A.S. Milton & E. Schönbaum. S. Karger, Basel, pp. 120–125.

Membrane effects of some chemical irritants on the giant amoeba (*Chaos carolinense*)

R.W. FOSTER, A.H. WESTON & K.M. WESTON

Department of Pharmacology, Materia Medica and Therapeutics, The University, Manchester M13 9PT

The ability of certain chemicals to irritate sensory neurones has lead to their use as rubefacients and as riot-control agents. Their mechanism of action remains unknown despite investigation using a variey of mammalian preparations. We have tested the effects of certain irritants on the electrophysiological properties of the giant amoeba, *Chaos carolinense*, to explore their mechanism and assess its suitability as a model of the mammalian sensory irritation process.

The resting membrane potential and input resistance of *Chaos carolinense* in modified Pringsheim's solution (Chapman-Andresen, 1962) were 111.5 ± 0.52 mV and 9.6 ± 0.3 Mohm respectively (mean $\pm$ s.e. mean, $n = 256$). Dibenzoxazepine (CR) produced a concentration-dependent decrease in input resistance (e.g. to 3.1 Mohm with 10^{-5} mol/l) but no significant change in resting membrane potential ($n = 6$). The effects of other chemicals tested are shown in Table 1. The relative potencies of these compounds do not reflect those found on testing in mammalian preparations (Ramage, 1977; N. Creasey, personal communication).

The cell membrane of *Chaos carolinense* behaved as though impermeable to Na^+ and Cl^- and permeable to K^+, as found previously by Riddle (1962) and Bruce & Marshall (1965). Using their values for the intracellular concentration of K^+, the distribution of K^+ across the membrane conforms to a Donnan equilibrium, the resting membrane potential being the K^+ equilibrium potential.

The decrease in input resistance seen after exposure to these irritants is interpreted as due to an opening of K^+ channels in the cell membrane. If such an event occurs in mammalian sensory neurones it would be unlikely to produce excitation and may therefore not be involved in any direct mode of action. However, capsaicin also produces a reversible non-specific loss of reactivity in a variety of mammalian preparations (Ginawi, 1979) and this could be due to increased K^+ permeability.

Table 1 Effects of some chemical irritants on membrane potential and input resistance of *Chaos carolinense*

	Conc (mol/l)	*Membrane potential (mV)*	*Input resistance (Mohm)*	*Log_{10} potency relative to CR (mean $\pm$ 95% C.I.)*
2-chloro-dibenzoxazepine[1] (2-ClCR)	5×10^{-6}	−114.5	4.9	-0.7 ± 0.4
3-chloro-dibenzoxazepine[1] (3-ClCR)	2×10^{-6}	−108	4.3	0.3 ± 0.3
n-nonanoyl-vanillylamine[1] (VAN)	2×10^{-4}	−109.3	7.2	-3.2 ± 0.9
capsaicin (CAP)	10^{-4}	−104.4	8.8	-2.7 ± 1.0
o-chloro-benzylidenemalononitrile[1] (CS)	2×10^{-4}	−104.5	3.7	-0.7 ± 0.9

$n = 8$. Conc = concentration. C.I. = confidence interval.

[1] These irritants were kindly supplied by the Chemical Defence Establishment, Porton Down.

References

BRUCE, D.L. & MARSHALL, J.M. (1965). Some ionic and bioelectric properties of the ameba Chaos chaos. *J. Gen. Physiol.*, **49**, 151–178.

CHAPMAN-ANDRESEN, C. (1962). Studies on pinocytosis in amoebae. *C.R. Trav. Lab. Carlsberg.*, **33**, 73–264.

GINAWI, O.T. (1979). The selectivity of capsaicin. M.Sc. Thesis, University of Manchester.

RAMAGE, A.G. (1977). An investigation of the effects of chemical irritants on sensory nerves in the cat skin. Ph.D. Thesis, University of Manchester.

RIDDLE, J. (1962). Studies on the membrane potential of *Pelomyxa carolinensis*. *Exp. Cell Res.*, **26**, 158–167.

Inhibition by naloxone of endotoxin-induced reactions in mice

D.J.M. WRIGHT & M.P.I. WELLER
(introduced by J.B.E. BAKER)

Departments of Medical Microbiology and Psychiatry, Charing Cross Hospital Medical School, Fulham Palace Road, London, W6 8RF

Holaday and Faden (1978) inhibited endotoxin-induced hypotension in rats with naloxone. In our experiments naloxone prevented the fall in temperature and circulating white blood cells and platelets in mice given endotoxin.

In both the mouse models used, there were six mice in each test group; for the controls and full description of the changes produced by endotoxin see Wright (1980).

In the first model, *Escherichia coli* lipopolysaccharide (100 μg) dissolved in saline, was given twice intravenously to six-week old inbred CBA/CA mice at spaced intervals, 20 h apart. After the second inoculation, there was an immediate fall in the number of circulating white blood cells and platelets, which was reversible in three hours. The defect of the above model was the inability to demonstrate the role of endotoxin in an infective speticaemia. The objection was overcome by studying the reaction after treating mice infected with *Borrelia duttoni* (Wellcome mouse strain) with ampicillin (10 mg) given intraperitoneally, the mice having been infected 2 days previously with a standard inoculum of Borrelia. Following treatment there was an acute fall in temperature, white blood cell and platelets with the release of endotoxin-like material into the peripheral circulation. These changes reverted to normal in three to five hours. This reaction is mediated by an endotoxin-like material which is contained in Borrelia (Wright, 1980).

Naxolone was dissolved and diluted to the appropriate concentration immediately before each experiment with sterile, non-pyrogenic physiological saline. The dose of the drug is expressed in terms of free base. Naloxone was given intraperitoneally 1 h before giving the second inoculation of lipopolysaccharide to normal mice or ampicillin to the infected mice.

The suppression of the endotoxin-induced reactions, seen in Table 1, need not be explained on the basis of endorphin inhibition as suggested by Holaday *et al.* (1978). Lemaire and his colleagues (1978) suggested on indirect evidence in rats, that naloxone blocks 5-HT

Table 1 The influence of naloxone on the response to either treating Borrelia infections or to challenge with a second inoculation of lipopolysaccharide

Agent tested	*Dose*	*One hour after second lipopolysaccharide inoculation*		*One hour after treatment of borrelial infections*	
		WBC count	*Platelet count*	*WBC count*	*Platelet count*
Saline		<8.4	<5.7	<16	<8
Naloxone	10.0 mg/kg	70.7 ± 7	86.5 ± 7.3	75.5 ± 14.7	73.6 ± 12.5
	1.0 mg/kg	98.7 ± 5	93.6 ± 6.6	68 ± 8	75 ± 7.6
	0.1 mg/kg	<8.4	<5.7	64 ± 12.8	79 ± 11.2
	0.01 mg/kg	<8.4	<5.7	<16	<8

The mean value of the pre-treatment WBC count was $8.0 \pm 0.7 \times 10^9/1$ and a pre-treatment mean platelet count was $409 \pm 12 \times 10^9/1$. The mean white blood cell and mean platelet counts following treatment or challenge is expressed as a percentage of the normal count. The values of the mean and s.e. mean represents the results of two experiments, each with six mice.

re-uptake and in our experiments we have paralleled this change in our models using the very specific non-tricyclic 5-HT re-uptake blocking drug, fluoxetine (Lemberger *et al.*, 1978). Alternatively, in our experiments the beta-blocking drug propranolol also inhibits this reaction. It is of interest that Tampier *et al.* (1977), like Charalampous & Askew (1974), have shown that the beta-blocking drug propranolol could displace naloxone binding in brain tissue by competition for the same receptors.

References

CHARALAMPOUS, K.D. & ASKEW, W.E. (1974). Effect of non-opiate antagonists on stereo specific opiate receptor binding of tritiated naloxone. *Res. Commun. Chem. Pathol. Pharmacol.*, **8**, 615–622.

HOLADAY, J.W. & FADEN, A.I. (1978) Naloxone reversal of endotoxin hypotension suggests role of endorphins in shock. *Nature, Lond.*, **275**, 450–451.

LEMAIRE, I., TSENG, R. & LEMAIRE, S. (1978). Systemic administration of beta-endorphin: Potent hypotensive effect involving serotonergic pathway. *Proc. Natl. Acad. Sci.*, **75**, 6240–6242.

LEMBERGER, L., ROWE, H., CARMICHAEL, R., OLDHAM, S., HORNG, J.S., BYMASTER, F.P., & WONG, D.T. (1978). Pharmacologic effects in man of a specific serotonin-reuptake inhibitor. *Science*, **199**, 436–437.

TAMPIER, L., ALPERS, H.S. & DAVIS, V.E. (1977). Influence of catecholamine-derived alkaloids and beta adrenergic blocking agents and stereo specific binding of ^{3}H naloxone. *Res. Commun. Chem. Pathol. and Pharmacol.*, **17**, 731–636.

WRIGHT, D.J.M. (1980). Reaction following treatment of murine borreliosis and Shwartzman-type reactions with borrelial sonicates. *Parasite Immunol.*, (in press).

Polymyxin B sulphate markedly reduces the effects of *E. coli* endotoxin in anaesthetized cats

BERNADETTE HUGHES, B.R. MADAN & J.R. PARRATT

Department of Physiology and Pharmacology, University of Strathclyde, Royal College, George Street, Glasgow G1 1XW

Recent studies have demonstrated that the cyclic polypeptide antibiotic polymyxin B sulphate prevents a number of the biological effects that result from the administration of gram-negative bacterial endotoxins (e.g. hyperthermia, diffuse intravascular coagulation; Corrigan & Bell, 1971; Van Miert & Van Duin, 1978).

In the cat, the response to the administration of a lethal dose of *E. coli* endotoxin consists of an acute phase (occurring within 1–3 min and manifested by a marked rise in pulmonary artery and transient decreases in systemic arterial pressure and myocardial contractility) and a delayed shock phase (characterized by systemic hypotension, a reduced stroke volume and a severe metabolic acidosis; Parratt, 1973). Previous unpublished work in this department has shown that polymyxin B (5 mg/kg), administered 1 min before endotoxin, abolished this acute phase. In view of these observations, we have investigated the effects of this antibiotic on the delayed shock phase in this experimental model.

Cats were anaesthetized and prepared as previously described (Parratt, 1973). The early increase in right atrial pressure (of 9 $\pm$ 1.3 mmHg) and the transient systemic hypotension resulting from *E. coli* administration (2 mg/kg, i.v.) were not observed in any cat pretreated with polymyxin B sulphate (5 mg/kg infused i.v. for 30 min beginning 5 min before endotoxin and a further 5 mg/kg injected 1 min before endotoxin).

Polymyxin B also appeared to prevent the onset of the delayed shock phase and markedly reduced its severity. In control (endotoxin alone) animals, blood pressure decreased from 131 $\pm$ 5 mmHg to 69 $\pm$ 12 over a 5 h period and arterial pH fell from 7.38 $\pm$ 0.02 to 7.137 $\pm$ 0.01; only one cat survived the 8 h observation period. In the polymyxin and endotoxin treated cats there was a fall in blood pressure during the first 2 h after endotoxin (from 141 $\pm$ 9 to 89 $\pm$ 8 mmHg) but pressure returned to control levels at 3 h and was maintained for a further 5 hours. There was no significant decrease in pH. All cats in this group (n = 11) were alive 8 h after giving the endotoxin. Polymyxin alone caused systemic hypotension during the 30 min infusion period (137 $\pm$ 9 mmHg to 110 $\pm$ 9 mmHg), pressure returning to control levels at 1 hour. This hypotension was presumably due to histamine release from mast cells (Parratt & West, 1957).

These results indicate that polymyxin not only abolished the acute circulatory responses to endotoxin but also prevents the onset and severity of the delayed shock phase. These effects may be due to its ability to bind chemically to the lipid A moiety of the lipopolysaccharide molecule (Morrison & Jacobs, 1976), thus reducing its biological activity. The dramatic improvement in survival seen in this study suggests that this antibiotic might prove useful in clinical

septic shock, where this is due to the gradual release of endotoxin into the circulation.

This work was supported by the Medical Research Council; Professor Madan was a Senior Commonwealth Fellow in the Department from April to June 1975. His present address is: Department of Pharmacology, University of Riyadh, Saudi Arabia.

References

CORRIGAN, J.J. & BELL, B.M. (1971). Endotoxin induced intravascular coagulation: Prevention with Polymyxin B sulphate. *Jnl. Lab. Clin. Med.*, **77**, 802–810.

MORRISON, D.C. & JACOBS, D.M. (1976) Binding of polymyxin B to the lipid A portion of bacterial lipopolysaccharides. *Immunochemistry.* **13**, 813–818.

PARRATT, J.R. (1973). Myocardial and circulatory effects of *E. coli* endotoxin. Modification of responses to catecholamines. *Brit. J. Pharmac.*, **47**, 12–25.

PARRATT, J.R. & WEST, G.B. (1957). Action of polymyxin B on tissue mast cells of the rat. *J. Physiol. (Lond.)*, **135**, 24P.

VAN MIERT, A.S.J.P.A.M. & VAN DUIN, C.TH.M. (1977). The antipyretic effect of polymyxin B in endotoxin-induced fever. *Vet. Sci. Commun.*, **1**, 79–84.

Are inhibitory effects of anti-inflammatory steroids on chronic inflammation, acute oedema and cell accumulation in the rat mediated through a specific receptor?

J. COOTE, H.P. HUMPHRAY, I.F. SKIDMORE, P. SIBLEY, P.B. STEWART & B. WHITE
(Introduced by R.T. BRITTAIN)

Department of Biochemistry, Glaxo Group Research Limited, Ware, Hertfordshire, SG12 0DJ

Hormonal effects of steroids are exerted via binding to a class-specific cytosol receptor, stimulation of DNA transcription, RNA synthesis and protein synthesis (King, 1976; Schrader & O'Malley, 1977). These events provide criteria for defining whether or not the response to a steroid is specific or non-specific and we have used these criteria to analyse the anti-inflammatory action of steroids. In adjuvant arthritis, carrageenin oedema and carboxymethylcellulose-induced neutrophil accumulation in the rat a consistent rank order of potency: dexamethasone > triamcinolone > prednisolone > hydrocortisone can be established which is identical to the order of potency of these drugs in rheumatoid arthritis (Sarrett, Patchett & Steelman, 1963). A similar rank order of potency is obtained for steroid inhibition of plasminogen activator release from mouse macrophages, of prostaglandin production by mouse macrophages (Bray & Gordon, 1978) and rheumatoid synovial tissue (Robinson, McGuire, Bastian, Kantrowitz & Levine, 1978), of thromboxane A_2 production by guinea-pig perfused lung (Blackwell Flower, Nijkamp & Vane, 1978), for steroid induced thymus involution in rats and mice and for stimulation of glycogen accumulation in the livers of rats and mice (Sarrett, *et al.*, 1963). This consistent rank order suggests that all these effects are exerted via the glucocorticoid receptor.

Actinomycin D (2 mg/kg orally) given with the steroid prevented the inhibitory effect of dexamethasone (1 mg/kg orally) on carrageenin-induced oedema in rats but was ineffective when given 2.5 h after the steroid. These results are in agreement with those of Tsurufuji & Sugio (1978) who measured protein accumulation in an established carrageenin granuloma pouch. Actinomycin D (2 mg/kg orally) also prevented the inhibitory effect of dexamethasone (1 mg/kg orally) on neutrophil accumulation in a carboxymethylcellulose pouch in the rat. These results provide further evidence that the actions of anti-inflammatory steroids both on oedema and on cell emigration are exerted via a specific (glucocorticoid) receptor.

References

BLACKWELL, G.J., FLOWER, R.J., NIJKAMP, F.P. & VANE, J.R. (1978). Phospholipase A_2 activity of guinea-pig isolated perfused lungs: Stimulation and inhibition by anti-inflammatory steroids. *Br. J. Pharmacol.*, **62**, 79–89.

BRAY, M.A. & GORDON, D. (1978). Prostaglandin production by macrophages and the effect of anti-inflammatory drugs. *Br. J. Pharmacol.*, **63**, 635–642.

KING, R.J.B. (1976). Intracellular reception of steroid hormones. *Essays in Biochemistry*, **12**, 41–76.

ROBINSON, D.R., MCGUIRE, M.B., BASTIAN, D., KANTROWITZ, F.P. & LEVINE, L. (1978) The effects of anti-inflammatory drugs on prostaglandin production by rheumatoid synovial tissue. *Prostaglandins Med.* **1**, 461–477.

SARRETT, L.S., PATCHETT, A.A. & STEELMAN, S. (1963). The effects of structural alteration on the anti-inflammatory properties of hydrocortisone. *Progr. Drug Research*, **5**, 11–153.

SCHRADER, W.T. & O'MALLEY, B. (1977). Progesterone receptors of chick oviduct. *Adv. Exp. Med. Biol.*, **96**, 103–136.

TSURUFUJI, S. & SUGIO, K. (1978). Molecular mechanism in the manifestation of anti-inflammatory activity of glucocorticoids. *Eur. J. Rheumatol. Inflamm.*, **1**, 226–231.

Prolactin secretion and the postpartum blues syndrome

A.J. GEORGE, J.R.M. COPELAND & K.C.M. WILSON

**Pharmacology Department, School of Pharmacy, Liverpool Polytechnic. University Department of Psychiatry, Royal Liverpool Teaching Hospital, Liverpool*

Abnormal serum prolactin levels have recently been associated with premenstrual neurotic disorders (Mattson & Schoultz, 1974) and neurotic anxiety (Mathew *et al.*, 1979). This study investigated the relationship between serum prolactin and the components of the puerperal neurotic syndrome known as the postpartum 'blues'. Forty-two pregnant women with no previous history of mental illness were chosen for the study. Each subject was interviewed on the second, fourth and sixth days postpartum using the Present State Examination (P.S.E.) as modified by Cooper *et al.* (1977) for trained interviewers. A blood sample was obtained before the first breast-feeding of the day and before interview from each subject. Serum prolactin levels were measured, blind, by the radio-immunoassay method of Hwang *et al.* (1971). The assay standards and random samples were re-assayed against M.R.C. standards. Thirty-eight complete sets of blood samples and questionnaires were obtained.

Examination of the P.S.E. interviews showed that tension, anxiety, worries and depression (with its somatic symptoms) were the main features of the post-partum blues in terms of individual patients' scores when measured over the six day period. When the scores in each of these four categories were analysed in relation to serum prolactin levels it was found that there was a significant correlation between each of the components of the 'blues' and the serum prolactin level on each of the three days of the study (with the exception of depression on day six). (Table 1.)

No significant correlation was found between any of the psychiatric variables and breast feeding.

These results demonstrate that there is a significant relationship between serum prolactin and each of three mood variables over the six days postpartum and over four days for depression. It is suggested that prolactin or its central aminergic regulating system is associated with neurotic puerperal disorders.

The authors wish to thank Mr. G. Stevens for statistical advice. This study was financed by the Mersey Regional Health Authority. K.C.W. was supported by the R.A.M.C.

References

COOPER, J.E., COPELAND, J.R.M., BROWN, G.W., HARRIS, T. & GOURLAY, A.J. (1977). Further studies on interviewer training and inter-rater reliability of the Present State Examination. *Psychol. Med.*, **7**, 517–523.

HWANG, P., GUYDA, H. & FRIESEN, H. (1971). A radioimmunoassay for human prolactin. *Proc. Nat. Acad. Sci.* (Wash), 68, 1902–1906.

MATHEW, R.J., HO, B.T., KRALIK, P. & CLAGHOM, J.L. (1979). Anxiety and serum prolactin. *Am. J. Psychiat.*, **136**, 716–717.

MATTSON, B. & SCHOULTZ (1974). A comparison between lithium, placebo and a diuretic in premenstrual tension. *Acta. Psychiat. Scand.* (Suppl.), **255**, 75–84.

Table 1

	Serum prolactin level		
	Day 2	*Day 4*	*Day 6*
Anxiety	r = 0.56**	r = 0.44**	r = 0.41**
Tension	r = 0.54**	r = 0.42**	r = 0.33*
Worries	r = 0.48**	r = 0.46**	r = 0.42**
Depression and somatic symptoms	r = 0.43**	r = 0.39**	r = 0.29 N.S.
$n = 38$			

Significance Level * $P < 0.05$; ** $P < 0.01$.
Coefficients of correlation between each of four categories of mood and plasma prolactin level over the six days post partum.

Ovarian function in the rat following irreversible inhibition of ornithine decarboxylase with α-difluoromethylornithine

J.R. FOZARD,* J. GROVE & N.J. PRAKASH

Centre de Recherche Merrell International, 16, rue d'Ankara 67084 Strasbourg-Cedex, France

L-Ornithine decarboxylase (E.C. 4.1.1.17; ODC) forms putrescine from L-ornithine and is a key enzyme in the biosynthesis of the polyamines, spermidine and spermine (Tabor & Tabor, 1976). In normal rats with a four day oestrus cycle, the ODC activity in the ovary undergoes a transient, but substantial, rise late in pro-oestrus and only at that time (Kobayashi, Kupelian & Maudsley, 1971). The control of the induction of ODC by luteinizing hormone (LH) and its strategic positioning between the release of LH and ovulation (Kobayashi *et al.*, 1971; Kaye, Icekson, Lamprecht, Gruss, Tsafriri & Lindner, 1973) has led to repeated suggestions of a functional role for the enzyme in the early phase of LH action on the ovary (Kobayashi *et al.*, 1971; Williams-Ashman, Jänne, Coppoc, Geroch & Schenone, 1972; Guha & Jänne, 1976). The recent development of the irreversible, highly selective inhibitor of ODC, DL-α-difluoromethyl-ornithine (RMI 71782; DFMO) (Metcalf, Bey, Danzin, Jung, Casara & Vevert, 1978), has allowed us to investigate the functional role of the ODC induced in the rat ovary immediately preceding ovulation.

Female Sprague–Dawley rats exhibiting at least two consecutive 4 day cycles were injected s.c. with DFMO (500 mg/kg) at 12.00 on the day of pro-oestrus and 200 mg/kg given 6, 12 and 18 h later. Control animals were injected with saline. Some animals were killed to provide tissues for measurement of ODC activity and polyamine concentrations (Prakash, Schechter, Grove & Koch-Weser, 1978). Others were mated overnight (pro-oestrus-oestrus) with male rats of proven fertility. A final group was injected as above and mated 4 days later at the onset of the next pro-oestrus.

In confirmation of the data of Kobayashi *et al.* (1971) ovarian ODC activity increased markedly on the evening of pro-oestrus to reach a peak at 20.00 some 20 times higher than the early pro-oestrus value. The putrescine concentration also increased to reach a peak of six times the early pro-oestrus value at 23.00. Ovarian spermidine and spermine concentrations changed little during this period. Treatment with DFMO completely suppressed the pre-ovulatory increases in the ovarian ODC activity and putrescine concentration.

Animals treated with DFMO during pro-oestrus-oestrus and concomitantly housed with experienced males mated normally. The resulting pregnancies were also normal as assessed by the numbers and weights of the foeti, placentae and resorption nodules removed at autopsy on day 19 of gestation. Uteri from animals treated with DFMO during pro-oestrus-oestrus and mated 4 days later contained significantly more implantation sites (16.9 ± 1.0, mean ± s.e. mean, $n = 8$) than those from saline-treated control animals (14.1 ± 0.4, $n = 14$).

Thus, the pre-ovulatory rise in ovarian ODC appears not to be an essential feature of ovulation in that particular cycle. However, the possibility of a role for ODC in the initiation of development of the follicles which reach maturity in the succeeding cycle would be consistent with our data and remains the subject of our further study.

We thank Mrs M.L. Part and Mrs M. Nagy for expert technical assistance.

References

GUHA, S.K. & JÄNNE, J. (1976). Decarboxylation of ornithine and adenosylmethionine in rat ovary during pregnancy. *Acta Endoc.*, **81**, 793–800.

KAYE, A.M., ICEKSON, I., LAMPRECHT, S.A., GRUSS, R., TSAFRIRI, A. & LINDNER, H.R. (1973). Stimulation of ornithine decarboxylase activity by luteinizing hormone in immature and adult rat ovaries. *Biochemistry*, **12**, 3072–3076.

KOBAYASHI, Y., KUPELIAN, J. & MAUDSLEY, D.V. (1971). Ornithine decarboxylase stimulation in rat ovary by luteinizing hormone. *Science*, **172**, 379–380.

METCALF, B.W., BEY, P., DANZIN, C., JUNG, M.J., CASARA, P. & VEVERT, J.P. (1978). Catalytic irreversible inhibition of mammalian ornithine decarboxylase (E.C. 4.1.1.17) by substrate and product analogues. *J. Am. Chem. Soc.*, **100**, 2551–2553.

PRAKASH, N.J., SCHECHTER, P.J., GROVE, J. & KOCH-WESER, J. (1978). Effect of α-difluoromethylornithine, an enzyme-activated irreversible inhibitor of ornithine decarboxylase, on L 1210 leukemia in mice. *Cancer Res.*, **38**, 3059–3062.

TABOR, C.W. & TABOR, H. (1976). 1,4-Diaminobutane (putrescine), spermidine, and spermine. *A. Rev. Biochem.*, **45**, 285–306.

WILLIAMS-ASHMAN, H.G., JÄNNE, J. COPPOC, G.L., GEROCH, M.E. & SCHENONE, A. (1972). New aspects of polyamine biosynthesis in eukaryotic organisms. *Advances in Enzyme Regulation*, **10**, 225–245.

Apomorphine and gastric emptying in the guinea-pig

BARRY COX & CHRISTINE ENNIS

Department of Pharmacology, Materia Medica and Therapeutics, The Medical School, Manchester University, M13 9PT

Drugs which have in common the ability to interact with dopamine receptors also modify the rate of gastric emptying. We have investigated the effects of apomorphine on the rate of gastric emptying in the guinea-pig using a simple, non-invasive, X-ray fluoroscopic method. 25 polystyrene coated barium sulphate pellets (1 mm dia) were administered via an oesophageal cannula at 9.30 a.m. to guinea-pigs which had been allowed free access to food and water overnight. Immediately after feeding all the pellets were visible within the stomach. The time course of the pellets leaving the stomach was followed for 3 hours. Since there appeared to be a diurnal variation (Table 1) all experiments were performed in the morning.

Apomorphine (0.125 mg/kg and 0.5 mg/kg) injected 1 h after oral feeding produced a dose related inhibition of gastric emptying (Table 1) with the higher dose producing almost complete inhibition. The effects of two anti-emetic drugs known to block dopamine receptors and to increase the rate of gastric emptying in man were also investigated. Both compounds had no significant effect at 1 mg/kg but at 5 mg/kg produced a significant increase in gastric emptying (Table 1). However, higher doses (10 mg/kg) were inhibitory. Thus there was no clear dose-response relationship.

Metoclopramide (5 mg/kg) but not domperidone (5 mg/kg) prevented the inhibition of gastric emptying induced by apomorphine (0.125 mg/kg). Since domperidone does not readily penetrate the brain (Laduron & Leysen, 1979) the interaction between metoclopramide and apomorphine could be within the central nervous system. This is supported by the observation that only metoclopramide prevented signs of stereotypy. However, the mechanism by which domperidone increases gastric emptying still requires explanation. The site of action of domperidone seems likely to be outside the central nervous system, but whether or not it involves a dopamine receptor remains to be determined.

C.E. is an SRC CASE award scholar. The financial assistance of Janssen Pharmaceutica is gratefully acknowledged.

Reference

LADURON, P.M. & LEYSEN, J.E. (1979). Domperidone, a specific *in vitro* dopamine antagonist, devoid of *in vivo* central dopaminergic activity. *Biochem. Pharmacol.*, **28**, 2161–2166.

Table 1 Effect of drugs on the rate of gastric emptying in the guinea-pig

	Mean % pellets leaving stomach			
	1st Hour	2nd Hour	3rd Hour	*n*
Saline (9 a.m.)	16	14	29	22
Saline (2 p.m.)	14	36	10	6
Apomorphine				
0.125 mg/kg	—	7*	5*	6
0.5 mg/kg	—	1**	10*	6
Metoclopramide				
1 mg/kg	26	2*	30	8
5 mg/kg	11	52*	14	10
10 mg/kg	9	6	2*	6
Metoclopramide 5 mg/kg + Apomorphine 0.125 mg/kg	—	16	22	6
Domperidone				
1 mg/kg	23	18	8	6
5 mg/kg	13	44**	26	10
10 mg/kg	3	0**	5*	6
Domperidone 5 mg/kg + Apomorphine 0.125 mg/kg	—	2*	10	6

Significantly different from controls using the non-parametric Mann-Whitney U test, 2 tailed, * $P < 0.05$ ** $P < 0.01$.

SK&F 92994: a new histamine H_2-receptor antagonist

R.C. BLAKEMORE, T.H. BROWN, G.J. DURANT, J.C. EMMETT, C.R. GANELLIN, M.E. PARSONS & A.C. RASMUSSEN

The Research Institute, Smith Kline and French Research Ltd., Welwyn Garden City, Hertfordshire

SK&F 92994 (2-[2-(5-methyl-4-imidazolylmethylthio)-ethyl-amino]-5-(3,4-methylenedioxybenzyl)-4-pyrimidone dihydrochloride) is an example of a new type of histamine H_2-receptor antagonist which is more lipophilic than cimetidine (Brimblecombe, Duncan, Durant, Emmett, Ganellin & Parsons, 1975) [P(octanol-water at pH 8.5 and 37.5°C) 130, Mitchell, personal communication; cf. cimetidine, 2.5] and contains a 5-substituted isocytosine moiety in place of the cyanoguanidine group.

On the guinea-pig isolated atrium SK&F 92994 antagonised the positive chronotropic action of histamine but was slow to reach equilibrium (60 min compared to 8 min for cimetidine) and was difficult to wash out of the tissue. A pA_2 value could not be established since the slope of the Schild plot was significantly different from unity (0.74 ± 0.22 mean $\pm$ 95% confidence limits). However a dose ratio of two was obtained at a concentration of 0.2 μM (cf. cimetidine 3.2 μM). Concentrations of SK&F 92994 up to 100 μM had no significant effect on the response to isoprenaline, suggesting specificity for H_2 receptors.

SK&F 92994 antagonised the inhibitory action of histamine on the rat isolated uterus. Although the slope of the Schild plot (1.57 ± 0.73) was not significantly different from unity, the 95% confidence limits were very wide and at high concentrations the compound inhibited the twitch response elicited by electrical stimulation. A dose ratio of two was obtained at 0.056 μM (cf. cimetidine 2.7 μM).

SK&F 92994 had relatively weak but competitive H_1 receptor blocking activity on the guinea-pig ileum; pA_2 value = 5.44 (5.14–5.70) with a Schild plot slope of 1.11 (0.89–1.32) corresponding to a dose ratio of two at 10.3 μM. Equilibration on this tissue was within two minutes.

SK&F 92994 inhibited histamine-stimulated gastric acid secretion in a dose-related manner; in the perfused stomach preparation of the anaesthetised rat 50% inhibition was obtained at a dose of 0.092 (0.019–0.212) μmol/kg i.v. (n = 13), compared to 1.37 μmol/kg i.v. for cimetidine. Thus in this preparation SK&F 92994 was approximately fifteen times more potent than cimetidine and, in addition, the duration of the inhibitory action was prolonged.

In the conscious Heidenhain pouch dog the potency relative to cimetidine was approximately eight, a dose of 0.5 μmol/kg i.v. giving 61% inhibition (n = 6). (cf. cimetidine 68% at 4 μmol/kg i.v.). However, there was no significant difference in duration of action of the two compounds in this species. After oral administration to the dog, SK&F 92994 (5 and 10 μmol/kg) gave mean peak inhibitions of 53% and 92% respectively (n = 6) (cf. cimetidine 82% at 20 μmol/kg), suggesting that SK&F 92994 was some two to three times more potent by this route.

SK&F 92994 is a selective H_2-receptor histamine antagonist *in vitro* and a potent inhibitor of histamine-induced gastric acid secretion *in vivo* but its potency and duration of action relative to cimetidine is clearly dependent on the experimental circumstances. Since this may reflect underlying differences in the distribution and handling of the drug, studies in humans would be of interest.

Reference

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.

Inhibition by cimetidine of cardiac stimulation due to mast cell degranulation

R.W. GRISTWOOD, D.A.A. OWEN & I.R. SMITH

Department of Pharmacology, Smith Kline & French Research Limited, Welwyn Garden City, Hertfordshire

In the working guinea-pig heart *in vitro* histamine produces changes in cardiac function including increases in sinus rate, ventricular contractility and cardiac output (Flynn, Gristwood & Owen, 1979). The guinea-pig heart contains histamine in large quantities, at least a large proportion of which is stored within mast cells (Giotti, Guidotti, Mannaioni & Zilletti, 1966).

In the present study we have investigated histamine release and myocardial response due to mast cell degranulation in the working guinea-pig heart *in vitro*. Guinea-pigs of weight 250–300 g were sensitised

to ovalbumin (100 mg i.p. plus 10 mg s.c.) and at least three weeks later isolated working hearts were set up and cardiac parameters recorded as previously described (Flynn, Gristwood & Owen, 1978). In a number of preparations surface electrocardiograms were recorded differentially between right atrium and left ventricle and the signal stored on magnetic tape for retrieval and analysis. Coronary flow samples (0.5 ml) were collected immediately before and at 30 s intervals after ovalbumin challenge (4 mg bolus over 5 s) and these assayed for histamine using a modification (S-Adenosyl-L-[methyl-^{3}H]-methionine as methyl group donor with [U-^{14}C]-histamine to correct for efficiency of methylation and extraction) of the enzymatic double isotope histamine assay (see Beaven & Horakova, 1978).

Ovalbumin challenge evoked the release of histamine, peak release rate 18.0 ± 0.7 μg $min^{-1}g^{-1}$ dry wt ($n = 5$), at 30 s. Accompanying histamine release were large increases in sinus rate (119 ± 6 beats/min), ventricular contractility (2124 ± 183 mmHg/s) and aortic flow (179 ± 16 ml $min^{-1}g^{-1}$), and a decrease in coronary flow (20.1 ± 3.3 ml $min^{-1}g^{-1}$), all ($n = 8$). In addition to the tachycardia, arrhythmias occurred including premature ventricular contractions (25.5 ± 7, $n = 4$), during the first 3 min, and heart block (≥2°) which persisted for up to 10 min after challenge.

Histamine release in the presence of cimetidine (3.16×10^{-5} M) was similar to control (23.5 ± 4.8 μg min^{-1} g^{-1} at 30 s, $n = 4$). Cimetidine however, abolished the increase in ventricular contractility and significantly reduced the increases in sinus rate (64.2 ± 7.1 bts/min, $n = 6$) $P < 0.001$ and aortic flow (39 ± 10 ml min^{-1} g^{-1}, $n = 6$), $P < 0.001$. Cimetidine also significantly reduced both the number of premature ventricular contractions (1.75 ± 1.75, $n = 4$), $P < 0.02$ and the extent of heart block. The coronary flow response remained unchanged.

Mepyramine (10^{-7} M) did not affect histamine release or reduce the cardiac stimulation which occurred after ovalbumin challenge.

The results indicate that myocardial mast cell degranulation results in histamine release with concomitant changes in cardiac function. The ability of cimetidine significantly to reduce the cardiac stimulation indicates that the histamine released from mast cells stimulates histamine H_2-receptors and that this interaction is largely responsible for the cardiac stimulation observed during mast cell degranulation.

References

BEAVEN, M.A. & HORAKOVA, Z. (1978). In *Handbook of Experimental Pharmacology*, vol **XVIII/2** Ed. M. Rocha e Silva; Springer Verlag, Berlin, pp. 151–173.

FLYNN, S.B., GRISTWOOD, R.W. & OWEN, D.A.A. (1978). Characterisation of an isolated working guinea-pig heart including effects of histamine and noradrenaline. *J. Pharmac. Meth.*, **1**, 183–195.

FLYNN, S.B., GRISTWOOD, R.W. & OWEN, D.A.A. (1979). Differentiation of the roles of histamine H_1- and H_2-receptors in the mediation of the effects of histamine in the isolated working heart of the guinea-pig. *Br. J. Pharmac.*, **65**, 127–137.

GIOTTI, A., GUIDOTTI, A., MANNAIONI, P.F. & ZILLETTI, L. (1966). The influences of adrenotropic drugs and noradrenaline on the histamine release in cardiac anaphylaxis *in vitro*. *J. Physiol. (Lond.)*, **184**, 924–941.

Unaltered lymphocyte β-adrenoreceptor response in hypo- and hyperthyroidism

J.H. SILAS, B.M. SMITH & R.O. YATES

University Department of Therapeutics, Royal Hallamshire Hospital, Sheffield S10 2JF

Altered β-adrenoceptor responsiveness may be responsible for some of the clinical features of hypo- and hyperthyroidism (Malbon, Moreno, Cabelli & Fain, 1978). However, cardiovascular responses to isoprenaline infusion were not altered in patients suffering from thyrotoxicosis or myxoedema (McDevitt, Riddell, Hadden & Montgomery, 1978). Clinical studies, however, are unable to examine β-adrenoreceptor responses in isolation.

Lymphocytes have been shown to contain β_2-adrenoreceptors which respond to isoprenaline stimulation with an increase in cyclic AMP (cAMP) generated (Conolly & Greenacre, 1977). We have studied β-adrenoreceptor responsiveness of peripheral blood lymphocytes in relation to thyroid dysfunction.

Blood was collected from the following:

(a) Six patients before treatment for thyrotoxicosis and again when they were euthyroid.

(b) Six patients before and after treatment for myxoedema.

(c) Six 'control' patients without thyroid disease.

Table 1 Mean ± s.e. mean basal cAMP concentration (pmol/10^6 cells) and mean increase in cAMP following stimulation with isoprenaline

Patients	*Basal*	*Isoprenaline conc. (M)* 10^{-9}	10^{-8}	10^{-7}	10^{-6}
Controls	3.47 ± 0.42	1.64 ± 0.48	7.30 ± 1.12	13.88 ± 1.93	14.30 ± 2.45
Untreated thyrotoxicosis	4.94 ± 0.46*	1.42 ± 0.36	7.31 ± 1.94	14.03 ± 2.08	12.17 ± 2.20
Treated thyrotoxicosis	4.77 ± 1.20	2.12 ± 0.71	9.25 ± 3.01	14.16 ± 3.98	14.54 ± 4.31
Untreated myxoedema	3.11 ± 0.50	1.71 ± 0.35	6.17 ± 0.69	12.79 ± 1.21	13.44 ± 1.62
Treated myxoedema	4.05 ± 0.91	1.72 ± 0.41	5.66 ± 1.40	12.01 ± 1.82	12.50 ± 2.03

* Higher than controls ($P < 0.05$) and untreated myxoedema ($P < 0.025$).

Lymphocytes were isolated from venous blood and incubated for 15 min with either isoprenaline (10^{-9}–10^{-6} M) or ascorbic acid 'blank'. The method was similar to that described by Conolly & Greenacre (1977). The concentration of cells in the incubation medium was determined before incubation by visual counting and the sample diluted to give a suspension of 2–5 × 10^6 cells/ml. The reaction was terminated by rapid centrifugation and by the addition of ethanol −0.1 N HCl (80:20). The content of cAMP in the supernatant was assayed (Brown, Albano, Ekins & Sgherzi, 1971). Results were expressed as pmol cAMP/10^6 cells. The coefficient variation of the entire procedure was 8–12%.

Although basal cAMP concentrations were higher in untreated thyrotoxicosis than in normal controls and in untreated myxoedema (unpaired *t*-test) the response to stimulatio with isoprenaline was similar in all groups (Table 1). It is concluded that human lymphocyte β-adrenoreceptor responsiveness is not altered in hypo- or hyperthyroidism.

References

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

CONOLLY, M.E. & GREENACRE, J.K. (1977). The β-adrenoceptor of the human lymphocyte and human lung parenchyma. *Br. J. Pharmac.*, **59**, 17–23.

MALBON, C.C., MORENO, F.J., CABELLI, R.J. & FAIN, J.N. (1978). Fat cell adenylate cyclase and β-adrenergic receptors in altered thyroid states. *J. Biol. Chem.*, **253**, 571–678.

MCDEVITT, D.G., RIDDELL, J.G., HADDEN, D.R. & MONTGOMERY, D.A.D. (1978). Catecholamine sensitivity in hyperthyroidism and hypothyroidism. *Br. J. Clin. Pharmac.*, **6**, 297–302.

The activity in man of a new aldosterone antagonist (SC27169)

G.T. McINNES, J.R. SHELTON, M.J. ASBURY, I.R. HARRISON, L.E. RAMSAY, J.M. CLARKE & G.R. VENNING

Department of Medicine, Gardiner Institute, Western Infirmary, Glasgow G11 6NT and
Division of Clinical Research, G.D. Searle & Co. Ltd., High Wycombe, Bucks., HP12 4HL

SC27169 (17-hydroxy-3-oxo-17α-pregn-4-ene-7α, 21 dicarboxylic acid, 7-(1 methyl ethyl) ester, monopotassium salt) has properties consistent with competitive mineralocorticoid antagonism *in vitro* (Funder, Feldman, Highland & Edelman, 1974) and in animals where it is at least equipotent to spironolactone. We examined the activity of single doses of SC27169 in man and compared its potency relative to spironolactone in two studies using methods depending on urinary electrolyte responses to mineralocorticoid antagonists in the presence of fixed doses of the agonist, fludrocortisone (Ramsay, Harrison, Shelton & Tidd, 1975; McInnes, Asbury, Shelton, Harrison, Ramsay, Venning & Clarke, 1979).

Study one was in three phases and involved six volunteers. Treatments compared were SC27169, spironolactone (each 50 mg) and placebo. The second was a six-phase study of twelve volunteers. Treatments tested were SC27169 (50 mg, 100 mg and 200 mg) and

Table 1 Mean and s.e. mean results for urinary electrolyte excretion 2–10 h after administration of placebo, SC27169 and spironolactone

	Placebo	*SC27169* 50 mg	*SC27169* 100 mg	*SC27169* 200 mg	*Spironolactone* 25 mg	*Spironolactone* 50 mg	*Spironolactone* 100 mg
Study 1 ($n = 6$)							
Volume (ml)	456 (83)	387 (54)				423 (53)	
Sodium excretion (mmol)	14.7 (3.3)	31.8 (8.0)*				26.0 (3.4)	
Potassium excretion (mmol)	20.9 (1.9)	15.3 (2.1)*				11.9 (2.5)†	
$Log_{10}10$ Na/k	0.80 (0.07)	1.28 (0.05)†				1.39 (0.11)†	
* $P < 0.05$ versus placebo							
† $P < 0.01$ versus placebo							
Study 2 ($n = 12$)							
Volume (ml)		541 (54)	538 (69)	636 (53)	552 (65)	560 (62)	628 (66)
Sodium excretion (mmol)		19.4 (3.2)″	25.9 (2.4)″	40.9 (5.0)″	19.1 (6.9)′	23.7 (2.7)′	30.8 (3.5)′
Potassium excretion (mmol)		19.2 (1.9)	19.9 (1.2)	19.4 (1.8)	21.0 (1.4)	18.6 (2.0)	17.7 (1.4)
Log_{10} 10 Na/k		0.973 (0.051)″	1.103 (0.028)″	1.318 (0.041)″	0.941 (0.053)″	1.100 (0.043)″	1.220 (0.062)″

Significance of the slope of the log dose-response trends ′ $P < 0.005$, ″ $P < 0.001$.

spironolactone (25 mg, 50 mg and 100 mg). Both studies were of double-blind, balanced, crossover design, phases being separated by weekly intervals. Fludrocortisone was administered at 1900 h and test medications at 2100 h. Overnight urine was collected.

In the first study, SC27169 showed significant activity for sodium excretion ($P < 0.05$), potassium excretion ($P < 0.05$) and $\log_{10}$ 10 Na/K ($P < 0.01$). Spironolactone showed activity only for potassium excretion ($P < 0.01$) and $\log_{10}$ 10 Na/K ($P < 0.01$) (Table 1). SC27169 and spironolactone did not differ significantly. For urinary $\log_{10}$ 10 Na/K, the best single index of renal mineralocorticoid (Johnson, 1954) and antimineralocorticoid activity (Kagawa, 1964), the potency of SC27169 relative to spironolactone was estimated at 0.60 with 95% confidence limits of 0.24–1.42, using an approach described by McInnes, Asbury, Shelton, Harrison, Ramsay, Venning & Clarke (1979).

In the second study (Table 1) criteria for valid estimation of the potency of SC27169 relative to spironolactone were satisfied for sodium excretion and $\log_{10}$ 10 Na/K. Using $\log_{10}$ 10 Na/K, relative potency was 0.61 (95% confidence limits 0.48–0.79).

SC27169 exhibited significant renal antimineralocorticoid activity but, in the more stringent second study, it was significantly less potent than spironolactone. This is in contrast to the results of animal experiments which have consistently suggested that SC27169 was at least as potent as spironolactone. The relative potency estimate derived from the simpler protocol of the first study showed excellent agreement with that from the formal parallel line bioassay of the second study.

References

FUNDER, J.W., FELDMAN, D., HIGHLAND, E. & EDELMAN, I.S. (1974). Molecular modification of anti-aldosterone compounds: Effects on affinity of spirolactones for renal aldosterone receptors. *Biochem. Pharmacol.*, **23**, 1493–1501.

JOHNSON, B.B. (1954). Bioassay of adrenal cortical steroids on the basis of electrolyte excretion by rats. Effects of 11-desoxy and 11-oxy steroids. *Endocrinology*, **54**, 196–208.

KAGAWA, C.M. (1964). Adrenocortical antagonists. In: Evaluations of Drug Activities. Pharmacometrics. Eds. Laurence, D.R. and Bacharach, *A.L. Academic Press Inc.* (New York).

MCINNES, G.T., ASBURY, M.J., SHELTON, J.R., HARRISON, I.R., RAMSAY, L.E., VENNING, G.R. & CLARKE, J.M. (1979). The active constituent of spironolactone after acute administration in man. Proceedings of the British Pharmacological Society, London.

RAMSAY, L., HARRISON, I., SHELTON, J. & TIDD, M. (1975). Relative potency of prorenoate and spironolactone in normal man. *Clin. Pharmac. Ther.*, **18**, 391–400.

Enhancement of hydrocortisone absorption by actively transported solutes in the jejunum in man

G.I. SANDLE, M.J. KEIR & C.O. RECORD
(introduced by M.D. RAWLINS)

Gastroenterology Unit and Department of Medical Physics, Royal Victoria Infirmary and University of Newcastle upon Tyne

Intestinal absorption of hydrocortisone occurs by passive diffusion (Schedl & Clifton, 1961). Glucose is actively transported and has been shown to increase hydrocortisone absorption in both the jejunum and the ileum in man, possibly by increasing mucosal cellular metabolism (Schedl & Clifton, 1963). We have further investigated this using galactose and alanine, both of which are actively transported, but neither of which are metabolised locally by the mucosa.

Using a double-lumen tube technique, 20 cm segments of proximal jejunum were perfused (flow rate 15 ml/min) in healthy subjects ($n = 5$) with normal saline containing polyethylene glycol 4000 5 g/litre, first without, then with 100 mg/litre hydrocortisone hemisuccinate plus 25 μCi/litre of titriated hydrocortisone to allow measurement of steroid absorption. Other subjects were similarly perfused with saline containing 56 mmol/litre glucose (Glu; $n = 5$), galactose (Gal; $n = 4$) or alanine (Ala; $n = 4$), first without and then with, hydrocortisone.

We found: (1) hydrocortisone absorption (% absorption ±s.e. mean) from Glu (77.9 ± 5.2), Gal (70.3 ± 8.7) and Ala (68.0 ± 7.3) was greater than from normal saline (46.3 ± 6.3; $P < 0.0025$, $P < 0.05$ and $P < 0.05$ respectively; (2) with normal saline, water movement (ml/10 min ± s.e. mean; + = absorption, − = secretion) did not change significantly ($0.15 < P < 0.2$) from +5.49 ± 8.68 in the absence, to −0.83 ± 6.4 in the presence of hydrocortisone. However, hydrocortisone significantly altered water movement from +25.21 ± 10.60 to +46.18 ± 10.64 ($0.0025 < P < 0.005$) with Glu, from +31.3 ± 5.8 to +49.9 ± 10.6 ($0.0125 < P < 0.025$) with Gal, and from +27.9 ± 11.1 to +46.8 ± 6.4

($0.025 < P < 0.05$) with Ala; (3) there was a significant correlation ($r = 0.656$, $P < 0.0025$) between absorption of hydrocortisone and water; (4) hydrocortisone did not significantly increase glucose, galactose or alanine absorption.

Thus, intra-luminal hydrocortisone increased water absorption in the presence of actively transported solutes. Consequently, increased water absorption may have facilitated the passive absorption of hydrocortisone by solvent drag.

References

SCHEDL, H.P., CLIFTON J.A. (1961). SMALL INTESTINAL ABSORPTION OF STEROIDS. *Gastroenterology*, **41**, 491–499.

SCHEDL, H.P. & CLIFTON, J.A. (1963). Cortisol absorption in man. *Gastroenterology*, **44**, 134–145.

Modification of cardiovascular effects of histamine infusions in man by chlorpheniramine and cimetidine

M.J. BOYCE, D.A.A. OWEN & K. WAREHAM

The Research Institute, Smith Kline & French Research Limited, Welwyn Garden City, Hertfordshire, AL7 1EY

Animal studies have shown species variation in the cardiovascular responses to histamine (Owen, 1977). We have studied the cardiovascular effects of 5 min intravenous infusions of histamine before and after chlorpheniramine (10 mg i.v.) and cimetidine (200 mg i.v.), singly and in combination, in six healthy volunteers on three separate days. Systolic and diastolic BP and heart rate were measured by an automatic sphygmomanometer (Arteriosonde) and heart rate meter, respectively, at half minute intervals. The presence and distribution of flushing of the skin was recorded.

Before the antagonists, histamine (20, 40 and 80 μg kg^{-1} h^{-1}) caused dose dependent falls in systolic and diastolic BP and increases in heart rate. The distribution of flushing, which was observed in all subjects throughout the histamine dose range, was also dose-dependent.

Areas under the curves for BP and heart rate were calculated and results subjected to analysis of variance. There were no significant differences between the three control dose-response curves. Chlorpheniramine and cimetidine, singly, each significantly ($P < 0.05$) reduced the histamine (20, 40, and 80 μg kg^{-1} h^{-1}) dose-responses. Chlorpheniramine tended to attenuate early changes in systolic and diastolic BP and heart rate whereas cimetidine tended to attenuate late changes in diastolic BP and heart rate. Flushing was abolished in four subjects during the lower histamine dose after chlorpheniramine compared with two after cimetidine. Each antagonist also attenuated flushing during the higher histamine doses.

Chlorpheniramine and cimetidine, in combination, abolished all responses to 20 μg kg^{-1} h^{-1} histamine and permitted two further doses of 160 and 320 μg kg^{-1} h^{-1} to be given, thereby restoring the control dose-responses. Flushing was abolished in four subjects and one subject during 40 and 80 μg kg^{-1} h^{-1} histamine doses, respectively.

The changes in BP and flushing are consistent with interaction of histamine at H_1- and H_2-receptors in peripheral blood vessels. The heart rate changes are also consistent with interaction at H_1- and H_2-receptors probably causing direct myocardial stimulation as observed *in vitro* (Gristwood, Lincoln and Owen, 1980) and an indirect component, e.g. secondary to the fall in BP.

Short infusions of histamine were relatively well tolerated. The responses were reproducible and the method should permit further pharmacological characterisation of histamine receptors in the human cardiovascular system.

References

OWEN, D.A.A. (1977). Histamine receptors in the cardiovascular system. *Gen. Pharmacol.*, **8**, 141–156.

GRISTWOOD, R.W., LINCOLN, J.C.R. & OWEN, D.A.A. (1980). Effects of histamine on human isolated heart muscle: Comparison with effects of noradrenaline. *J. Pharm. Pharmac.*, (in press).

Chenodeoxycholic acid: Effects on the clearance of antipyrine and isoniazid in man

P. FARROW, S.A. McG. THOM, M.D. RAWLINS & OLIVER JAMES

Wolfson Unit of Clinical Pharmacology, The University, Claremont Place, Newcastle upon Tyne NE1 4LP, and the Department of Medicine and Geriatrics, Freeman Hospital, Newcastle upon Tyne

Chenodeoxycholic acid (CDCA) inhibits the activity of hydroxymethylglutaryl coenzyme A reductase (HMG CoA reductase), which may contribute to its cholesterol gallstone dissolving action (Coyne, Marks & Schoenfield, 1977). It has also been suggested that CDCA may enhance the activity of hepatic microsomal oxidation (Budillon, D'Arienzo, Mazzacca, Parrilli & Capuano, 1978). In order to investigate the possible interaction between CDCA and other drugs we have studied its effects on antipyrine and isoniazid clearances.

Antipyrine and isoniazid clearances were measured on separate occasions in 11 healthy volunteers before and after 3 week's treatment with 750 mg or 1000 mg per day CDCA. Antipyrine clearance was estimated (gas liquid chromatography) following an oral dose of 600 mg, using six salivary samples taken over 32 hours. Isoniazid clearance was measured (by spectrophotometry) after an oral dose of 10 mg/kg body weight, using four blood samples withdrawn over the next six hours. There was no significant difference between the antipyrine clearances before (mean 3.14 l/h $\pm$ s.e. mean 0.43 l/h) of after (mean 3.17 l/h $\pm$ s.e. mean 0.34 l/h) CDCA treatment. However mean isoniazid clearance increased significantly by 18.5% (Wilcoxon T = 7, $P = 0.02$).

Because of its differential actions on hepatic metabolism of cholesterol and bile acids the effects of 3 week's treatment with cholic acid (750–1000 mg/day) on antipyrine and isoniazid clearances were studied in an additional 7 volunteers. As with CDCA there was no change in antipyrine clearance before cholic acid treatment (mean 3.05 l/h $\pm$ s.e. mean 0.42 l/h) and after (mean 3.50 l/h $\pm$ s.e. mean 0.59 l/h). But unlike CDCA, cholic acid treatment did not affect isoniazid clearance, the mean change being +1.4% (Wilcoxon T = 14 N.S.).

Since CDCA at therapeutic doses does not affect hepatic microsomal oxidation of antipyrine, a significant interaction with other drugs metabolised by this route appears unlikely; furthermore, microsomal induction is improbable as a mode of action of CDCA in gallstone dissolution. The effects of CDCA in isoniazid clearance may occur as a result of the increased availability of acetyl CoA due to inhibition of HMG CoA reductase. However, the magnitude of the change in acetylation with CDCA is unlikely to be of major clinical importance.

References

BUDILLON, G., D'ARIENZO, A., MAZZACCA, G., PARRILLI, G. & CAPUANO, G. (1978). D-glucaric acid secretion during chenodeoxycholate therapy for gallstones. *Lancet*, **1**, 206.

COYNE, M.J., MARKS, J. & SCHOENFIELD, L.J. (1977). Mechanism of cholesterol gall-stone formation. *Clinics in Gastroenterology*, **6**, 1, 129–139.

The effect of increased caffeine intake on the metabolism and pharmacokinetics of theophylline in man

J. CALDWELL, C.A. LAWRIE & T.J. MONKS

Department of Biochemical and Experimental Pharmacology, St. Mary's Hospital Medical School, London W2 1PG

Previous work from this laboratory has shown that the metabolism of theophylline in man involves one pathway, *N*-demethylation to 3-methylxanthine, which is saturated by doses of 100 mg (Monks, Caldwell & Smith, 1979). When a drug's elimination involves saturable processes, it is to be expected that it will exhibit dose-dependent pharmacokinetics (Rowland, 1976). In the case of theophylline this is hard to study directly, since the tea, coffee and related foods in the usual Western diet lead to the accumulation of a body pool of methylxanthines chemically related to theophylline, between which exist complex metabolic interrelationships. We have therefore approached this problem of dose dependency by manipulation of dietary methylxanthines, and have shown that removal of the body pool of these leads to faster and more extensive metabolism of theophylline (Monks *et al.*, 1979). Since the body pool of methylxanthines inhibits theophylline metabolism, it might be expected that augmenting this pool will have a further inhibitory effect. Accordingly, we have examined the effect of increased caffeine intake on the fate of theophylline in man.

Three healthy male laboratory workers (ages 24–30 years, wt. 68–88 kg) who had participated in our previous study (Monks *et al.*, 1979), consumed in addition to their usual intake of tea, coffee and other methylxanthine-containing foods 6 12 oz bottles of Diet-Pepsi (each containing 45 mg caffeine) daily for 9 days. On the 8th day, they received an i.v. infusion of ^{14}C-theophylline (100 mg; 10 μCi) and collected their urine hourly for 8 h, two hourly from 8–24 h and at 36 and 48 h. Analysis of urinary metabolites and calculation of kinetic parameters was as described by us (Monks *et al.*, 1979). In addition to theophylline (13% of dose in 24 h), three major metabolites were excreted, 3-methylxanthine (17%), 1,3-dimethyluric acid (33%) and 1-methyluric acid (14%), and two minor unknowns (total 2%) were also present. The elimination of all of these followed apparent first-order kinetics with the exception of 3-methylxanthine, whose elimination followed Michaelis-Menten kinetics. When these results were compared with those previously obtained in the same subjects, the only difference seen was that the excretion of unchanged theophylline rose from 8% of dose on the usual diet to 13% on the caffeine-supplemented diet. This increase is the only result consistent with expectation, but is most likely due not to inhibition of metabolism but to the increase in urine flow from 1.0 ml/min on the usual diet to 2.2 ml/min as a result of caffeine supplementation (mean values). It is known that the excretion of theophylline is urine flow dependent (Levy & Kaysooko, 1976).

Consideration of the kinetic parameters of theophylline metabolism by these subjects suggests that the normally present pool of methylxanthines inhibits 3-methylxanthine formation maximally, and that further increasing its size will have little effect. The data reported here confirm this expectation.

Supported by the Asthma Research Council. T.J.M. was an MRC research student. We are grateful to Prof. R.L. Smith for providing facilities.

References

LEVY, G. & KAYSOOKO, R. (1976). Renal clearance of theophylline in man. *J. Clin. Pharmacol.*, **16**, 329–332.

MONKS, T.J., CALDWELL, J. & SMITH, R.L. (1979). Influence of methylxanthine containing foods on theophylline metabolism and kinetics. *Clin. Pharmacol. Ther.*, **26**, 513–524.

ROWLAND, M. (1976). Pharmacokinetics. In *Drug Metabolism from Microbe to Man* Ed. Parke, D.V. & Smith, R.L. pp. 121–145. Taylor & Francis, London.

Inter-individual differences in the conjugation of paracetamol with glucuronic acid and sulphate

J. CALDWELL, S. DAVIES & R.L. SMITH

Department of Biochemical and Experimental Pharmacology, St. Mary's Hospital Medical School, London, W2 1PG

The minor analgesic paracetamol is well-known to undergo metabolic conjugation with glucuronic acid and sulphate, two reactions which have an important bearing upon the inactivation, clearance and toxicity of the drug (Jollow & Smith, 1977). Little information is available concerning inter-individual variation in the relative utilisation of these two pathways, a factor which influences responsiveness to the drug. Accordingly, we have undertaken a preliminary investigation of the inter- and intra-subject variation in man of the relative extents of glucuronic acid and sulphate conjugation of paracetamol.

34 healthy volunteers (25 M, 9 F), aged 20–28 years, each took by mouth 1 g paracetamol (2 × 500 mg tablets with 250 ml water) early morning following a light breakfast, and collected their 0–8 h urine. Each sample was then assayed for paracetamol, paracetamol sulphate and paracetamol glucuronide as described by Caldwell, Smith & Davies (1978).

The results showed that there occurred considerable inter-individual variation in the ability to form the glucuronic acid and sulphate conjugates. The average total recovery of paracetamol was 78% of dose (range 64–90), which was made up of 7% as free paracetamol (range 2–16), 34% as its glucuronide (range 13–60) and 38% as its sulphate (range 14–52). The relative extents of the two conjugation pathways for each individual, expressed as the ratio of the % total recovery as the glucuronide to the % total recovery as the sulphate (the G/S ratio) ranged from 0.26–4.28. This ratio was not normally distributed (Kurtosis = 8.37) the frequency distribution showing a marked positive skew (2.76) (i.e. some individuals showed a relative inability to form the sulphate conjugate). The G/S ratio was positively correlated with the total recovery of paracetamol in the urine by the Spearman rank test ($r_{(s)} = +0.40$; $P < 0.05$). There was a positive rank correlation between the % total recovery as sulphate and total recovery ($r_{(s)} = 0.35$;

$P < 0.05$) and a poorer negative rank correlation between % total recovery as glucuronide and total recovery ($r_{(s)} = -0.21$; $P < 0.23$). These data imply that a metabolic pattern involving extensive sulphate conjugation facilitates a high total excretion of paracetamol in 8 h, whereas the converse is true for extensive glucuronidation. In further studies, 10 of the subjects were studied on a second occasion, and the relative extents of sulphate and glucuronic acid conjugation were reproducible within a maximum range of ±18%. Thus, the magnitude of intra-subject variation was far less than that of inter-subject variation.

S.D. is grateful to the Wellcome Trust for a student vacation study award.

References

CALDWELL, J., SMITH, R.L. & DAVIES, S.A. (1978). Drug metabolism in a case of progeria. *Gerontology*, **24**, 373–380.

JOLLOW, D. & SMITH, C.A. (1977). Biochemical aspects of toxic metabolites: formation, detoxication and covalent binding. in *Biological Reactive Intermediates* Ed. Jollow, D., Kocsis, J.J., Snyder, R. & Vainio, H. pp. 42–61, Plenum, New York.

Haem utilization by rat liver tryptophan pyrrolase as a screening test for drug exacerbation of hepatic porphyrias

A. A.-B. BADAWY

South Glamorgan Health Authority, Addiction Unit Research Laboratory, Whitchurch Hospital, Cardiff CF4 7XB, Wales

The haem-biosynthetic pathway is normally controlled in such a manner that there is no excessive accumulation or excretion of porphyrins or their precursors. The porphyrias are a group of conditions (hepatic and erythropoietic) in which this control is lost, and abdominal pains and neurological disorders are the most prominent symptoms (for a review, see Beattie & Goldberg, 1972–1974). In this group of diseases, particularly hepatic porphyrias, therapeutic intake of a variety of drugs precipitates an acute attack, and although known exacerbators of the disease are usually avoided, there is no available means of predicting whether a new drug is likely to precipitate an attack. Work from this laboratory (Badawy, 1978) suggests that haem utilization by rat liver tryptophan pyrrolase under conditions involving the potentiation of experimental hepatic porphyria may provide the first possible screening test for drug exacerbation of the porphyrias.

Rat liver tryptophan pyrrolase has been shown to be the most sensitive hepatic haemoprotein for assessing delicate changes in liver haem concentration (for a review, see Badawy, 1979). Haem regulates its own synthesis by a feedback mechanism(s) exerted at the rate-limiting step of the pathway, catalyzed by 5-aminolaevulinate synthase. Accordingly, administration of the experimental porphyrogen 3,5-diethoxycarbonyl-1,4-dihydrocollidine (DDC) causes a marked enhancement of synthase activity by producing an early depletion of pyrrolase (and other haemoprotein) haem. The porphyria produced by DDC is potentiated by joint administration of phenylbutazone (De Matteis & Gibbs, 1972). Under these conditions, the potentiation of the DDC enhancement of synthase activity is accompanied by a further early depletion of pyrrolase haem (Badawy, 1978).

A further early depletion of pyrrolase haem is produced in DDC-treated rats by any of the following exacerbators of hepatic porphyrias: apronalide, chlordiazepoxide, chloroquine, chlorpropamide, dichlorophenazone, diphenylhydantoin (phenytoin) ergotamine, ethanol, glutethimide, griseofulvin, meprobamate, α-methyldopa, oestradiol, phenobarbitone, progesterone, sulphanilamide, thiopentone and tolbutamide. By contrast, none of the following nonexacerbators of the disease causes a further early depletion of pyrrolase haem: cortisol, glipizide, imipramine, isocarboxazid, ketamine, mefenamic acid, morphine, oxpentifylline, paracetamol (acetaminophen), pethidine, pheniramine, propranolol and sodium salicylate.

The following is a summary of the test. Groups (5 each) of male Wistar rats (150 g) are starved for 24 h before death. A 50 mg/kg dose of DDC or an equal volume (1 ml/kg) of the solvent dimethylformamide is given i.p. 4.25 h, whereas a dose of the test drug or an equal volume (10 ml/kg) of the solvent arachis oil is given p.o. 4 h, before death. Pyrrolase activity is measured in liver homogenates in either the absence (holoenzyme) or the presence (total enzyme) of added haematin (Badawy & Evans, 1975). The apoenzyme activity, calculated by difference, is used to estimate the haem-saturation ratio (holoenzyme/apoenzyme). Haem depletion is indicated by the decrease in this ratio.

The following are the findings with an exacerbator (progesterone, 2 mg/kg) and a nonexacerbator (cortisol acetate, 2 mg/kg) of porphyria. The activities of the pyrrolase holoenzyme and total enzyme (in μmol of kynurenine formed/h per g wet wt. of liver ± s.e. mean for each group of four rats) and the haem-satur-

ation ratio (derived from the above means) were respectively as follows: control (5.1 ± 0.49, 10.6 ± 1.3 and 0.93); DDC (3.5 ± 0.29, 10.5 ± 0.3 and 0.50); progesterone (5.1 ± 0.49, 10.5 ± 1.5 and 0.94); DDC + progesterone (2.4 ± 0.18, 9.8 ± 0.3 and 0.32); cortisol (5.2 ± 0.23, 12.9 ± 0.2 and 0.68); DDC + cortisol (3.5 ± 0.16, 10.9 ± 0.2 and 0.47).

This work was supported by the Wellcome Trust.

References

BADAWY, A.A.-B. (1978). Tryptophan pyrrolase, the regulatory free haem and hepatic porphyrias-early depletion of haem by clinical and experimental exacerbators of porphyria. *Biochem. J.*, **172**, 487–494.

BADAWY, A.A.-B. (1979). Central role of tryptophan pyrrolase in haem metabolism. *Biochem. Soc. Trans.*, **7**, 575–583.

BADAWY, A.A.-B. & EVANS, M. (1975). The regulation of rat liver tryptophan pyrrolase by its cofactor haem-experiments with haematin and 5-aminolaevulinate and comparison with the substrate and hormonal mechanisms. *Biochem. J.*, **150**, 511–520.

BEATTIE, A. & GOLDBERG, A. (1972–1974). The porphyrias. *Medicine*, **12**, 774–782.

DE MATTEIS, F. & GIBBS, A. (1972). Stimulation of liver 5-aminolaevulinate synthetase by drugs and its relevance to drug-induced accumulation of cytochrome *P*-450-studies with phenylbutazone and 3,5-diethoxycarbonyl-1,4-dihydrocollidine. *Biochem. J.*, **126**, 1149–1160.

First-pass propranolol uptake by dog lungs *in vivo*

J.A. PANG, J.P. BLACKBURN, R.J.A. BUTLAND & D.M. GEDDES (introduced by A.F. LANT)

Brompton Hospital and Westminster Hospital, London

In-vitro experiments show that propranolol is markedly concentrated in mammalian lung (Dollery & Junod, 1976). The most likely site is thought to be the pulmonary endothelial cell. This process may therefore reflect certain aspects of pulmonary endothelial function. In order to investigate this possibility we studied the effect of dose on the first-pass uptake of propranolol in ambulant dogs (body weight 19.5–31 kg) with catheters chronically implanted in the right atrium, pulmonary artery and aorta. In order to determine the influence of haemodynamics on the uptake of propranolol we also measured cardiac output and pulmonary artery pressure concurrently.

Propranolol uptake was estimated by comparing the ratio of [^{14}C]-propranolol to indocyanine green (ICG) injected into the right atrium with the ratio of their concentrations in aortic blood collected over the duration of the first-pass outflow dye curve. Collection was terminated before there was any visible dye recirculation. ICG was chosen because it does not leave the intravascular space and can therefore be used as a "marker" substance which was not removed by the lungs. Cardiac output was measured by standard dye dilution techniques. The dose of ICG used was 1.875 mg in all the experiments.

The percentage uptake at an injected dose of propranolol (0.02 mg) was 44 ± 14.4% (mean ± s.d., $n = 20$ in 5 dogs); at 0.2 mg, 44.3 ± 10.8% ($n = 44$ in 5 dogs); at 2 mg, 57.7 ± 11.4% ($n = 23$ in 6 dogs). Mean percentage uptake values at the first two doses were not significantly different, but the percentage uptake when propranolol (2 mg) was given was different from the first two ($P < 0.001$). There was no significant relationship between percentage uptake and cardiac output over the measured range (2.69–7.35 l/min; mean 4.84 l/min, s.d. 0.95 l/min, $n = 61$), nor was there any relationship between percentage uptake and pulmonary artery pressure. However, percentage uptake showed a tendency to fall with the number of days after catheterization. Possible explanation for these findings will be discussed.

In conclusion, it appears that first-pass percentage uptake of propranolol by dog lungs *in vivo* is constant at the lower dose but increases at a higher dose. It is independent of changes in cardiac output and fluctuation in pulmonary artery pressure, and may therefore be suitable as a means of studying the pulmonary endothelium.

Reference

DOLLERY, C.T. & JUNOD, A.F. (1976). Concentration of (±) propranolol in isolated, perfused lungs of rat. *Br. J. Pharmac.*, **57**, 67–71.

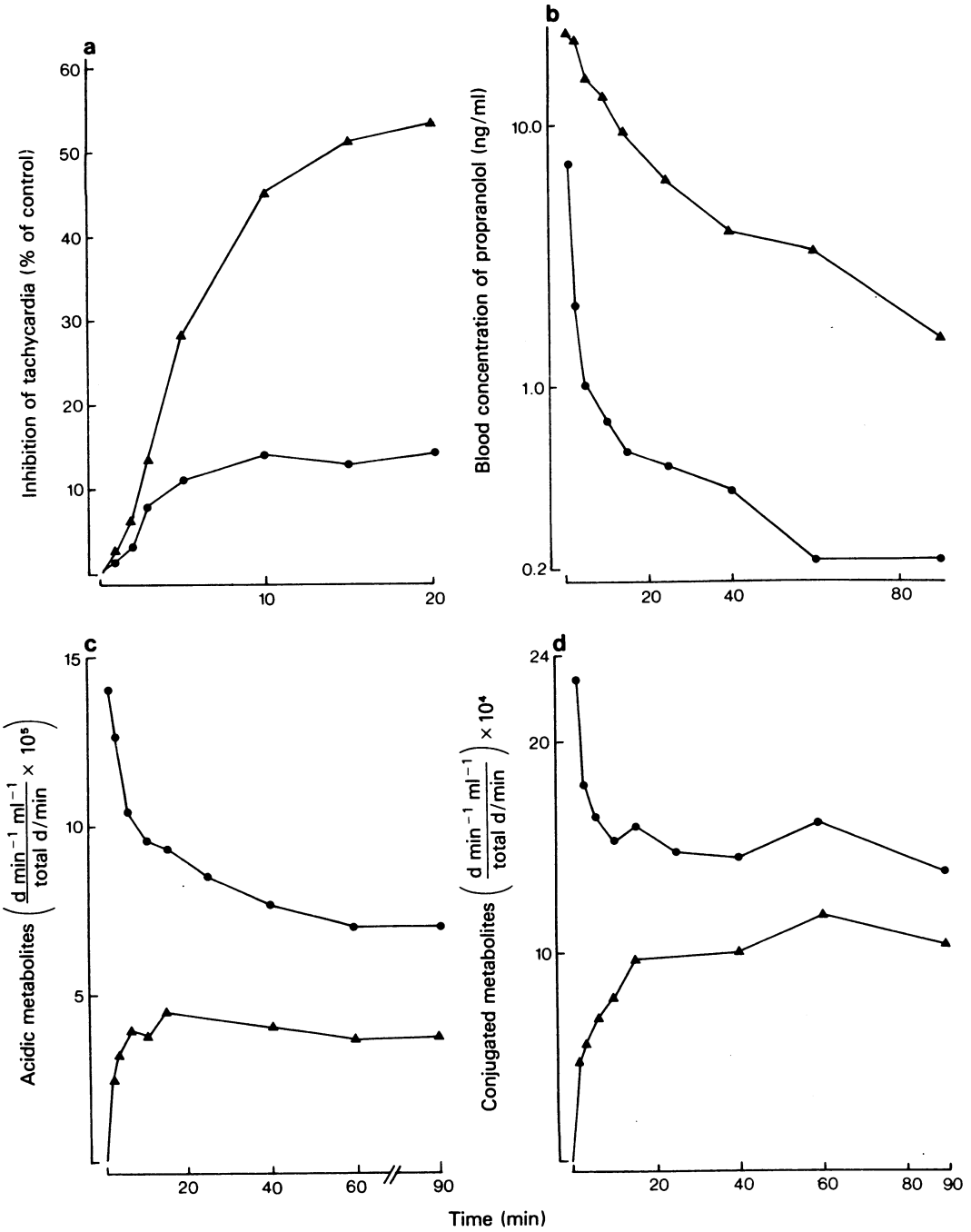


Figure 1 Illustrative results from a single rat showing inhibition of stimulation induced tachycardia (a), blood concentrations of propranolol (b) and formation of acidic (c) and conjugated metabolites (d) following the intra portal administration of propranolol (0.1 mg/kg) either alone (●) or with the co-administration of chlorpromazine (▲).

Reduction of the first pass elimination of propranolol by the co-administration of chlorpromazine

H.E. BARBER, N.R. KITTERINGHAM & J.C. PETRIE

Department of Pharmacology, and Therapeutics and Clinical Pharmacology, University Medical Buildings, Foresterhill, Aberdeen AB9 2ZD, Scotland

Some drugs are so extensively metabolised in either the intestinal wall, liver, or both of these tissues during the first passage through them that only a small fraction of the dose is available for therapeutic action. This is called the 'first pass effect' (F.P.E.). The pithed rat preparation (Gillespie, Maclaren & Pollock, 1970) has been used in a combined pharmacokinetic and pharmacodynamic study to investigate the F.P.E. of propranolol which has been reduced by competing for its liver uptake and/or metabolism by the co-administration of chlorpromazine.

Rats were pithed under halothane anaesthesia and ventilated with 100% oxygen. The pithing rod electrode was placed for optimal continuous stimulation of the sympathetic outflow to the heart (C6–T1, 0.05 ms, 1 Hz, 30–60 V,). [^{3}H]-Propranolol (0.1 mg/kg, 200 μCi/kg) was rapidly injected either via the jugular vein or a cannulated side branch of the hepatic portal vein. In some of the experiments chlorpromazine (loading dose = 4 mg/kg then 2.5 mg/kg/h) was infused via the portal venous route 45 min prior to and following the propranolol injections. Blood samples were obtained from one cartoid artery and analysed for propranolol and its metabolites using an HPLC method based on that of Pritchard, Schneck & Hayes (1979), arterial pressure was measured from the other carotid from which heart rate was obtained using a Grass tachograph.

Chlorpromazine administered intraportally inhibited the metabolism of propranolol resulting in a greater concentration of propranolol in the blood and a reduced concentration of metabolites (Figures 1, b, c and d). The higher concentrations of propranolol were associated with an increased percentage inhibition of the electrically induced tachycardia (Figure 1a). Chlorpromazine only had a small effect on the disposition and effectiveness of propranolol when given via the jugular route. Our results extend the observations of Vestal, Kornhause, Hollifield & Shand (1979) who reported a similar effect of chlorpromazine on the metabolism of propranolol which was associated with an increased hypotensive effect when the propranolol was administered chronically to man.

These results may have wider implications. If the F.P.E. of a drug can be reduced therapeutic effects could be obtained with lower doses thus decreasing the variability of response. If the metabolites were associated with undesirable side effects these could also be lessened when the dose was lowered.

This work was supported by I.C.I. Limited. We thank Dr. J. Cruickshank for his interest in the project.

References

GILLESPIE, J.S., MACLAREN, 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.

PRITCHARD, J.F., SCHNECK, D.W. & HAYES, A.H. (1979). Determination of propranolol and six metabolites in human urine by high pressure liquid chromatography. *J. Chromatog.*, **162**, 47–58.

VESTAL, R.E., KORNHAUSER, D.M., HOLLIFIELD, J.W. & SHAND, D.G. (1970). Inhibition of propranolol metabolism by chlorpromazine. *Clin. Pharmacol. Ther.*, **25**, 19–24.

Effect of enzyme induction on the pharmacokinetics of lignocaine and tocainide in rat

P.N. BENNETT, LIDIA J. NOTARIANNI & S. SUPRADIST

School of Pharmacy and Pharmacology, University of Bath

Tocainide (2-amino-2',6'-propionoxylidide) a structural analogue of lignocaine has recently been introduced as an antiarrhythmic agent suitable for oral administration to man. Although both drugs undergo extensive biotransformation in the liver, lignocaine has a high extraction ratio (ER) ($\simeq$0.99) and elimination is governed by liver blood flow (LBF), whereas tocainide has a low ER ($\simeq$0.25) and would be expected to exhibit metabolism dependent kinetics. Hepatic enzyme induction may thus produce differing effects on the kinetics of these drugs. Furthermore some enzyme inducing agents increase LBF (phenobarbitone, PB) whereas others do not (3,4 benzpyrene,

Table 1 Pharmacokinetic parameters following i.v. and oral administration of lignocaine and tocainide in control and 3.4-BP or PB treated rats (see text for details)

			Lignocaine			*Tocainide*	
(i)	*i.v.*	*n*	$T_{1/2}$ (min)[1]	AUC (μg/ml.min)[2]	*n*	$T_{1/2}$ (min)[1]	AUC (μg/ml.min)[2]
	control	8	48.1 ± 3.9	445 ± 48.2	5	128 ± 4.9	4002 ± 197
	3,4-BP	6	40.8 ± 5.0	345 ± 65.5	6	116 ± 14.4	2378 ± 105**
	PB	9	58.2 ± 10.2	227 ± 48.2*	6	67.2 ± 12.0*	2029 ± 239**
(ii)	*oral*	*n*			*n*		
	control	8	59.2 ± 4.6	103 ± 9.5	6	277 ± 19.7	4303 ± 544
	3,4-BP	6	54.5 ± 3.4	65.7 ± 7.6*	6	212 ± 9.8*	2191 ± 146*
	PB	6	62.0 ± 5.6	33.1 ± 7.9**	6	213 ± 6.1*	1828 ± 168*

Values are mean s.e. mean.
[1] $t_{\frac{1}{2}}$—terminal blood elimination half-life.
[2] AUC—area under blood concentration-time curve (time $0 \rightarrow \infty$).
Statistical significance of differences between test and control groups determined using Student's *t* test.
* $P < 0.01$; ** $P < 0.001$.

3,4-BP). The effects of treatment with 3,4-BP and with PB on the kinetics of lignocaine and tocainide have therefore been studied.

Male CFY rats (300–350 g) were treated with either sodium PB (100 mg/kg in saline, i.p.) for 4 days, or with a single dose of 3,4-BP (80 mg/kg in corn oil, i.p.), or with the same volume of saline or corn oil only. Rats prepared in this way were given lignocaine HCl (5 mg/kg, i.v. or 70 mg/kg, by mouth) or tocainide HCl (35 mg/kg, i.v. or 50 mg/kg, by mouth) and serial blood samples taken from tail vessels were assayed for the parent compound by GLC.

With both 3,4-BP and PB there was a significant increase in microsomal enzyme activity as determined by pentobarbitone sleeping time, hepatic microsomal protein and cytochrome P-450 content. Total LBF as measured by the radioactively labelled microsphere method of McDevitt and Nies (1976) was significantly increased after PB but was not significantly changed after 3,4-BP.

Table 1 shows that alteration in half-life and in AUC may occur as a result of enzyme induction and increased LBF. The data are compatible with the predictions of the perfusion-limited model of hepatic drug elimination (Rowland, Benet and Graham, 1973; Wilkinson and Shand, 1975) for changes in drug metabolism and LBF.

We are grateful to Astra Chemicals Ltd. for supporting this work.

References

McDevitt, D.G. & Nies, A.S. (1976). Simultaneous measurement of cardiac output and its distribution with microspheres in the rat. *Cardiovas. Res.*, **10**, 494–498.

Rowland, M., Benet, L.Z. & Graham, G.G. (1973). Clearance concepts in pharmacokinetics. *J. Pharmacokinet. Biopharmaceut.*, **1**, 123–136.

Wilkinson, G.R. & Shand D.G. (1975). A physiological approach to hepatic drug clearance. *Clin. Pharmacol. Therap.*, **18**, 377–390.

Effects of oxidative N-demethylation on mouse liver lysosomes and liver function

D.A. LEWIS & C.M. RAYMONT

The Pharmacological and Cancer Chemotherapy Laboratories, Department of Pharmacy, University of Aston in Birmingham, Birmingham B4 7ET

Certain compounds undergo oxidative N-demethylation in the liver to produce cytotoxic agents. The reasons for the cytoxic effects of the products of oxidative N-demethylation are not fully understood.

We have examined the effect of the antineoplastic triazenes, 5-(3,3-dimethyl-triazeno)imidazole-4-carboxamide (DTIC-Dome) and p-carboxymethylphenyldimethyltriazene (CMPDT), and the carcinogen dimethylnitrosoamine (DMNO) on the stability of lysosomes in isolated lysosome suspensions and on lysosomes in homogenates. Lysosomal stability was investigated by incubating the lysosomes with the various agents and

determining the amounts of acid phosphatase released in the supernatants (Weissmann & Thomas, 1963). All three agents stabilised the isolated liver lysosomes over a wide concentration range (10^{-2} M–10^{-6} M) but this effect was lost in homogenates where concentrations greater than 10^{-5} M lysed the lysosomes. When 5-(3-methyl-1-triazeno)imidazole-4-carboxamide (MTIC), a metabolite of DTIC-Dome, and p-carboxymethylphenylmonomethyltriazine (CMPMT), a metabolite of CMPDT, were tested by the same method no such differences were found. These metabolites do not undergo further demethylation and so it appears that the lytic action of the parent compounds was due to fragments of oxidative demethylation produced only in the homogenates.

As a study of *in vivo* liver function, the four compounds DTIC-Dome, MTIC,CMPDT and CMPMT were administered at 40 mg/kg i.p. to mice. Three days later their plasma was examined for the acute phase protein alpha$_1$-antitrypsin, as measured by its trypsin inhibiting capacity (T.I.C.) (Dietz, Rubinstein & Hodge, 1974). DTIC-Dome and CMPDT showed values of 127% ($P < 0.01$) and 120% ($P < 0.05$) respectively over controls. Their metabolites MTIC and CMPMT showed no significant elevation in T.I.C. values. DMNO elevated T.I.C. values in the rat (Lewis, Parrott, Bird and Best, 1979).

For histological studies DTIC-Dome was administered to mice i.p. (40 mg/kg) daily for 5 days. This dose has an antineoplastic effect. 1 h after the final dose 0.2 ml of Rhodamine conjugated mouse plasma was injected i.v. and the mice killed 1 h later. The livers were sectioned in a Bright's freezing cryostat (6 μ sections) and examined by ultra-violet microscopy. Dosed livers showed considerable diffusion of plasma into cells when compared with controls showing that permeability changes had occurred *in vivo*. Other sections stained with Sudan Black showed an accumulation of lipid droplets also indicative of permeability changes, and haematoxylin and eosin staining showed signs of necrosis.

A possible explanation for our results is that lysosomal damage *in vivo* may induce an acute inflammatory response as shown by the increased membrane permeability and the elevation in plasma T.I.C. values. This explanation warrants further investigation since lysosomal damage is associated with cancer (Poole, 1973) and tumour regression after chemotherapy (Allison, 1968).

C.M.R. is supported by an S.R.C. grant and we wish to thank Dr A. Gescher for helpful discussions, and Professor M.F.G. Stevens for providing the compounds.

References

ALLISON, A.C. (1968). The role of lysosomes in the action of drugs and hormones in *Advances in Chemotherapy*, **3**, 253–302. (Ed. A. Goldin, F. Hawking & R.J. Schnitzer) Academic Press, New York.

DIETZ, A.A., RUBINSTEIN, H.M. & HODGE, L.V. (1974). Measurement of alpha$_1$- antitrypsin in serum by immunodiffusion and by enzymatic assay. *Clin. Chem.*, **20**, 396–399.

LEWIS, D.A., PARROTT, D.P., BIRD, J. & BEST, R. (1979). Anti-inflammatory action following liver damage in the rat. *Agents & Actions* **9/2**, 201–204.

POOLE, A.R. (1973). Tumour lysosomal enzymes and invasive growth in *Lysosomes in Biology and Pathology*, **3**, 303–337 (Ed. J.T. Dingle) North Holland, Amsterdam.

WEISSMANN, G. & THOMAS, L. (1963). Studies on lysosomes 11. The effect of cortisone on the release of acid hydrolases from a large granule fraction of rabbit liver induced by an excess of vitamin A. *J. Clin. Invest.*, **42**, 661–669.

Structure of slow-reacting substances

H.R. MORRIS, PRISCILLA J. PIPER, MARWA N. SAMHOUN, G.W. TAYLOR & J.R. TIPPINS

Department of Biochemistry, Imperial College, London, S.W.7. and Department of Pharmacology, Royal College of Surgeons, London, WC2A 3PN

Slow-reacting substances (SRS) released immunologically and non-immunologically from various tissues are a family of compounds with potent pharmacological actions which are indistinguishable from one another (Morris, Piper, Taylor & Tippins, 1979a). Both SRS-A and rat basophil leukaemia (RBL-I) SRS are conjugated trienes (Morris, Taylor, Piper, Sirois & Tippins, 1978; Morris *et al.*, 1979a) derived from arachidonic acid by the action of lipoxygenase. All previous attempts to identify SRS-A have been unsuccessful, but recently the structure for an SRS from murine mastocytoma cells, leukotriene C, was described by Samuelsson at the Fourth International Prostaglandin Conference, Washington, 1979 (Fox, 1979). This has, however, since been modified (Samuelsson, Borgeat, Hammarstrom & Murphy, 1979) and the overall structure remains unknown.

SRS-A was prepared from lungs from guinea-pigs previously sensitised to ovalbumen as described by Engineer, Niederhauser, Piper & Sirois (1978). SRS

was obtained from RBL-I cells by a modification of the method of Jakschik, Falkenhein & Parker (1977). RBL-I cells were incubated at 37°C in a shaking water bath with indomethacin (1 μg/ml) and arachidonic acid (25 μg/ml) for 15 minutes. Cysteine (10^{-2} M) was added and the cells challenged 2.5 min later with calcium ionophore A23187 (10 μg/ml) and incubation continued for 20 minutes. The cells were precipitated by centrifugation at 1900 g and the supernatant extracted. At all stages of purification SRS-A or SRS were quantitated by bioassay on smooth muscle stripped from guinea-pig ileum (in the presence of mepyramine and hyoscine) against an in-house standard preparation of SRS-A.

After extensive purification including two high-pressure liquid chromatography (HPLC) steps (Morris, Taylor, Piper & Tippins, 1979b) SRS-A from guinea-pig lung can be fractionated into four related u.v. absorbing compounds (I–IV). The major area of biological activity corresponds to compound I (λ max 280 nm). SRS from rat basophil leukaemia (RBL-I) cells contains multiple peaks of bioactivity; one of which corresponds to compound I in terms of elution position ex-HPLC and u.v. spectrum (Morris, Piper, Taylor & Tippins, 1980).

We have now identified both the RBL-I SRS and guinea-pig SRS-A as peptidolipids (not leukotriene C) by using advanced spectrometric and protein chemical methods, and find that SRS and SRS-A are 5-hydroxy 6-cysteinylglycinyl, 7, 9, 11, 14 eicosatetraenoic acid. It is highly probable that human SRS-A has the same dipeptidyl structure (Morris, Piper, Taylor & Tippins, 1979a).

We wish to thank the Medical Research Council and the Asthma Research Council for grants. We also wish to thank Mr. David Gordon for valuable help with cell culture and Mr. M.A. Palmer for skilled technical assistance.

References

ENGINEER, D.M., NIEDERHAUSER, U., PIPER, P.J. & SIROIS, P. (1978). Release of mediators of anaphylaxis: inhibition of prostaglandin synthesis and the modification of release of slow-reacting substance of anaphylaxis and histamine. *Br. J. Pharmac.*, **62**, 61–66.

FOX, J.L. (1979). More roles for prostaglandins found. *Chem. & Eng. News.* **57**, (24) 19–22.

JAKSCHIK, B.A., FALKENHEIN, S. & PARKER, C.W. (1977). Precursor role of arachidonic acid in release of slow-reacting substance from rat basophilic leukemia cells. *Proc. Natl. Acad. Sci., U.S.A.*, **74**, 4577–4581.

MORRIS, H.R., PIPER, P.J., TAYLOR, G.W. & TIPPINS, J.R. (1979a). Comparative studies on immunologically and non-immunologically produced slow-reacting substances from man, guinea-pig and rat. *Br. J. Pharmac.*, **67**, 179–184.

MORRIS, H.R., TAYLOR, G.W., PIPER, P.J., SIROIS, P. & TIPPINS, J.R. (1978). Slow-reacting substance of anaphylaxis. Purification and characterisation. *Febs Letters*, **87**, 203–206.

MORRIS, H.R., TAYLOR, G.W., PIPER, P.J. & TIPPINS, J.R. (1979b). Slow-reacting substances of anaphylaxis: studies on purification and characterization. In *Prostaglandins and Inflammation*, AAS6. Ed. Rainsford, K.G. and Ford-Hutchinson, A.W. pp 27–36. Birkhäuser Verlag. Basel.

MORRIS, H.R., PIPER, P.J., TAYLOR, G.W. & TIPPINS, J.R. (1980). Structural investigations into slow-reacting substance of anaphylaxis (SRS-A) and related SRSs. (submitted for publication).

MURPHY, R.C., HAMMARSTROM, S. & SAMUELSSON, B. (1979). Leukotriene C: A slow-reacting substance from murine mastocytoma cells. *Proc. Natl. Acad. Sci. U.S.A.*, **76**, 4275–4279.

SAMUELSSON, B., BORGEAT, P., HAMMARSTROM, S. & MURPHY, R.C. (1979). Introduction of a nomenclature: Leukotrienes. *Prostaglandins*, **17**, 785–787.

A protective factor in guinea-pig urine against anaphylaxis

A. LEWIS & C.W.M. WILSON

Department of Pharmacology, University of Dublin, Trinity College, Dublin 2, Ireland

Male Duncan-Hartley guinea-pigs were immunized with ovalbumen dissolved in physiological saline (60 mg in 0.5 mls), in order to investigate whether urine provided any protective effect against anaphylaxis. During the course of two intestigations, plasma ascorbic acid (Roe & Kuether, 1943) and plasma glucose (Werner, Rey & Weillinger, 1970) were measured. In the second experiment, leucocyte ascorbic acid (Loh & Wilson, 1971) was also measured. In both experiments, five animals were used in each group and 48 hour urine specimens were collected from each animal. In the first experiment (I), guinea pigs were divided into control and experimental groups. Urine was collected from the experimental group on days 21 and 22 after the immunizing injection. On day 23, each animal received 2 mls of its own urine intraperitoneally (pre-challenge urine). Controls received saline. The injections were repeated on day 24. In the second experiment (2), five groups were used. Urine specimens were collected from groups A and B immediately after the immunizing dose of ovalbumen on days 1 and 2 (post-immunization urine) and from groups C and D on days 21 and 22 (pre-challenge

Table 1 Number of survivors and survival times of immunized guinea pigs protected with urine

Expt.	Control groups 1	Control groups 2	Boiled urine 2A (Post-imm)	Boiled urine 2C (pre-chal)	Unboiled urine 1 (pre-chal)	Unboiled urine 2B (post-imm)	Unboiled urine 2D (pre-chal)
Numbers surviving	0	0	1	0	4	4	2
Survival time (mins) of animals dying	7.8 ± 5.8	17.8 ± 5.5	48.3 ± 20.4	22.2 ± 9.5	25	80	44.0 ± 7.2
P values compared to controls	—	—	0.01	Not sig.	0.05	0.0005	0.0025

urine). All urine specimens were deep-frozen immediately after collection.

As in the first experiment, groups B and D received unboiled urine on days 23 and 24. Groups A and C received urine which had been boiled for 30 min prior to the injections. All the guinea-pigs received the challenge dose of ovalbumen on day 25. Group E also was challenged with ovalbumen but received physiological saline in place of urine. The three groups which received unboiled urine on day 23 developed muscle spasm and tremor which lasted for 1–5 mins. The control groups and groups receiving boiled urine showed no side effects. In the immunized guinea-pigs, plasma ascorbic acid increased and leucocyte ascorbic acid decreased significantly on day seven. Plasma ascorbic acid was reduced and leucocyte ascorbic acid rose significantly on day 21. In the survivors receiving unboiled urine, plasma ascorbic acid increased significantly 24 h after the challenge dose, accompanied by a fall in leucocyte ascorbic acid. Plasma glucose did not alter prior to the challenge dose of ovalbumen. Following the challenge dose, survivors receiving unboiled urine showed a significant increase in plasma glucose. The results indicate that a significant redistribution of ascorbic acid takes place 7 days after immunization. Up to 24 h after anaphylactic challenge, there was a radical alteration in ascorbic acid and glucose metabolism in the survivors. Injection of urine prior to challenge has a highly significant protective effect against anaphylaxis. In terms of survival time (Table 1), it appears that post-immunization urine may have more protective effect than pre-challenge urine. In that the protective factor was absent from boiled urine, it can be concluded that it is heat labile.

References

LOH, H.S. & WILSON, C.W.M. (1971). An improved method for the measurement of leucocyte ascorbic acid concentrations. *International J. Vit. and Nutr. Res.*, **41**, 90–98.

ROE, J.H. & KEUTHER, C.A. (1943). The determination of ascorbic acid in whole blood and urine through the 2,3-Dinitrophenylhydrazine derivative of ascorbic acid. *J. Biol. Chem.*, **147**, 399.

WERNER, W.H., REY, G. & WILLINGER, H. (1970). *Z. analyt. chem.*, **252**, 224.

Interactions between theophylline and theophylline derivatives in producing relaxation of guinea-pig isolated tracheal chains

LESLEY E. BOARDMAN
(Introduced by J. MORLEY)

Department of Clinical Pharmacology, Cardiothoracic Institute, Fulham Road, London SW3 6HP

Neobiphyllin, a combined formulation of theophylline (TP), monohydroxypropyl theophylline (MHPT) and dihydroxypropyl theophylline (DHPT), is used in the management of asthma. Although MHPT and DHPT are much less potent than TP in relaxing human bronchial smooth muscle *in vitro* (Svedmyr, 1977), there is some indirect clinical evidence to suggest that the combination produces synergistic rather than additive effects (Kamburoff & Loytved, 1979, Werdermann & Jorde, 1979).

Guinea-pig tracheal chains (Castillo & deBeer, 1947) were used to investigate possible drug interactions *in vitro*. Cumulative dose-response curves on

Table 1 Observed and predicted EC_{50} values for relaxation of tracheal chains by methylxanthines

Drug treatment	EC_{50} ($\times 10^{-4}$ M)									
	1	*2*	*3*	*4*	*5*	*6*	*7*	*8*	*Mean ± s.d.*	*Predicted*
TP	2.3	1.8	1.6	3.3	3.6	2.9	2.3		2.5 ± 0.7	
MHPT	4.3	4.1	5.5	4.3	8.5	5.9			5.4 ± 1.7	
DHPT	14.7	14.2	16.0	5.8	4.6	9.6			10.8 ± 4.9	
TP/MHPT	3.6	3.5	2.6	4.5	4.1	3.7			3.7 ± 0.7	4.0
TP/DHPT	6.2	3.3	4.3	3.8	7.5	4.1	4.2	6.1	4.9 ± 1.5**	6.7
MHPT/DHPT	7.3	7.9	10.3	7.4	7.7	5.3			7.6 ± 1.6**	8.1
TP/MHPT/DHPT	4.8	4.1	4.2	3.9	2.9	3.2			3.8 ± 0.7**	6.3

* $P < 0.01$.
** $P < 0.001$.

relaxation of histamine (4×10^{-6} M) contracted tracheal chains were constructed for the three methylxanthines administered alone and in all possible combinations. Since only three of the seven treatments could be tested on any single preparation, treatments were administered according to a randomised block design.

EC_{50} values (drug concentrations producing 50% maximal relaxation) were estimated from least squares linear regression analysis of the individual log concentration-response lines. Mean EC_{50}'s for each drug were used, firstly, to estimate the relative potencies of the three drugs and, secondly, to predict theoretical EC_{50} values for the combined drug treatments. A three way analysis of variance was undertaken to investigate the sources of variation and the significance of drug interaction.

Comparing the concentration-response curves for all treatments, the slopes were not significantly different and a maximal response of equal magnitude was achieved with each treatment. The relative potencies of TP, MHPT and DHPT were 3:5:11 respectively, as compared to ratios of 1:5:10 reported by Svedmyr (1977) who used isolated human tissue. Table 1 shows that, in all instances, the observed EC_{50}'s for combined drug treatments were less than the values predicted and that the synergistic interaction was highly significant for treatments TP/DHPT, MHPT/DHPT ($P < 0.001$) and TP/MHPT/DHPT ($p < 0.01$). These results show that the three methylxanthines administered in combination to isolated respiratory smooth muscle interact to produce an enhanced response.

This work was supported by H. Trommsdorff, West Germany, who also supplied the methylxanthines.

References

CASTILLO, J.C. & DEBEER, E.J. (1947). The tracheal chain. I. A preparation for the study of antispasmodics with particular reference to bronchodilator drugs. *J. Pharm. Exp. Ther.*, **90**, 104–109.

KAMBUROFF, P.L. & LOYTVED, G. (1979). Certain observations on the action of Neobiphyllin. *Int. Congress on Resp. Disease, Basel.*

SVEDMYR, N. (1977). The use of theophyllines in asthma therapy. *Scand. J. Resp. Dis.*, **Suppl. 101**, 13–24.

WERDERMANN, K. & JORDE, W. (1979). Zur bronchodilatatorischen wirkung von methylxanthinderivaten. *Dtsch. med. Wschr.*, **104**, 1307–1312.

Biologically active peptides in the skin of the plaice (*Pleuronectes platessa* L.)

A.A. ANDERSON, A.J. ASHRAF, THELMA C. FLETCHER[1] & G.M. SMITH

School of Pharmacy, Robert Gordon's Institute of Technology, Aberdeen and Institute of Marine Biochemistry, Aberdeen[1]

In a study of possible mediators of an immediate hypersensitivity reaction in the skin of plaice (*Pleuronectes platessa* L.), a marine teleost, prostaglandin E_2 was shown to be synthesised and released (Anderson, Fletcher & Smith, 1979). The reaction was not completely inhibited *in vivo* by indomethacin and therefore other mediators were sought.

During the isolation of a bradykinin-like component from the skin of plaice, another fraction was found which induced enhanced vascular permeability in rat skin. The levels of these components in the skin were not affected by prior injection of indomethacin (12 mg/kg wet weight of fish).

Sheets of skin from freshly killed plaice were incubated in methanol and extracted by the method used by Anastasi, Erspamer & Bertaccini (1965) to isolate bradykinin from frog skin. The concentrated extract from which the prostaglandins had been removed was taken up in ethanol, applied to an alumina column and eluted with ethanol of decreasing concentration (95%–20%). The extinction of the fractions was read at 280 nm. Aliquots were also hydrolysed with NaOH (Fruchter & Crestfield, 1965) and after neutralisation with acetic acid, colour developed with ninhydrin (Yemm & Cocking, 1955).

The concentrated fractions were assayed biologically on the following isolated preparations: rat stomach strip, duodenum, colon and uterus, guinea-pig ileum and rabbit aortic strip. Each fraction was also injected intracutaneously into the rat and the extravasation of intravenous Evan's blue used as a measure of activity (Harada, Takeuchi, Fukao & Katagiri, 1971).

Only the 60% ethanol fraction exhibited smooth muscle activity, with the rat uterus giving the most reproducible responses. The smooth muscle activity was completely abolished after incubation with trypsin or chymotrysin, or after dialysis. The fraction also caused relaxation of the rat duodenum. The properties of this fraction are not inconsistent with a bradykinin-like peptide. It was calculated from a 2 + 2 assay that the skin contained only 22.6 ng per g of tissue calculated as bradykinin equivalents. It is not known whether this kinin is generated from precursor proteins as in mammals, or if it exists preformed in the skin. Fractions eluted between 70% and 20% ethanol showed some dye leakage activity at equivalent protein content but 80% of the total activity was recovered from the 20% ethanol fraction, although this had no smooth muscle activity. The dye leakage activity was lost after dialysis.

All the fractions gave an erythematous response on intradermal injection into the plaice, but using a visual scoring method the 60% fraction was the most potent. However, we have not yet determined whether any of these fractions contain the primary mediators of the skin reaction.

AAA and AJA were supported by the Scottish Education Department and a grant from the S.R.C. is gratefully acknowledged.

References

ANASTASI, A., ERSPAMER, V. & BERTACCINI, G. (1965). Occurrence of bradykinin in the skin of *Rana Temporaria*. *Comp. Biochem. Physiol.*, **14**, 43–52.

ANDERSON, A.A., FLETCHER, T.C. & SMITH, G.M. (1979). The release of prostaglandin E_2 from the skin of plaice, *Pleuronectes platessa* L. *Br. J. Pharmac.*, **66**, 547–552.

FRUCHTER, R.G. & CRESTFIELD, A.M. (1965). Preparation and properties of two active forms of ribonuclease dimer. *J. Biol. Chem.*, **240**, 3868–3874.

HARADA, M., TAKEUCHI, M., FUKAO, T. & KATAGIRI, K. (1971). A simple method for the quantitative extraction of dye extravasated into the skin. *J. Pharm. Pharmac.*, **23**, 218–219.

YEMM, E.W. & COCKING, E. C. (1955). The determination of Amino-acids with Nihydrin. *Analyst.*, **80**, 209–214.

Tolerance to sodium cromoglycate in human lung fragments *in vitro*

KEVIN D. YOUNG & MARTIN K. CHURCH

Clinical Pharmacology, Faculty of Medicine, University of Southampton, Centre Block, Southampton General Hospital, Tremona Road, Southampton SO9 4XY

In rat mast cells, the anti-allergic activity of sodium cromoglycate is highly dependent on the time of drug administration. Cromoglycate is maximally effective in inhibiting histamine release when given simultaneously with antigen, but as the pre-incubation time with the tissue is extended the drug rapidly loses its inhibitory activity (Orr, Hall, Gwilliam & Cox, 1971; Sung, Saunders, Lenhardt & Chakrin, 1977). In contrast, cromoglycate inhibition of mediator release from human lung tissue *in vitro* does not decay rapidly with increasing pre-incubation time (Butchers, Fullarton, Skidmore, Thompson, Vardey & Wheel-

don, 1979). This communication reports that there is a loss of cromoglycate activity when human lung fragments are exposed to the drug over a prolonged period.

Human lung fragments were sensitised for 19 h with allergic serum, challenged with antigen (Timothy pollen extract) or anti-IgE and the histamine release measured, as described by Church & Gradidge (1978). A cromoglycate concentration of 2×10^{-4} M, which produced maximal inhibition in dose-response studies, was used throughout these experiments.

When cromoglycate was added 30 s before challenge, it inhibited antigen and anti-IgE induced histamine release by approximately 40%. Adding the drug 15 min before challenge produced similar inhibition. In a third group, cromoglycate was present throughout sensitisation and was renewed when the lung was washed free of serum, 15 min before challenge. This ensured continuous contact with the drug for 19–20 hours. Under these conditions, cromoglycate did not significantly inhibit either antigen or anti-IgE induced histamine release (5 experiments).

In contrast, salbutamol (10^{-6} M) inhibited histamine release by approximately 70% when preincubated with lung fragments for 30 s, 15 min or 19–20 hours. However, after prolonged contact with lung during the sensitisation period, the effects of salbutamol were not reversed by washing.

To study cross-tolerance, lung samples were exposed to cromoglycate throughout the 19 h sensitisation period, thoroughly washed and then challenged in the presence of salbutamol (10^{-7} M), chlorpromazine (10^{-4} M) or ICI 74917 (10^{-5} M). Prolonged preincubation with cromoglycate did not affect the inhibitory activity of salbutamol or chlorpromazine but did reduce that of ICI 74917.

In conclusion, prolonged contact of human lung with cromoglycate *in vitro* produces a tissue tolerance to the inhibitory effects of the drug. Such tolerance to drug activity is not seen with salbutamol or chlorpromazine but may be shared by drugs which are thought to act similarly to cromoglycate, such as ICI 74917.

This work was supported by the Chest, Heart and Stroke Association (K.D.Y.). Sodium cromoglycate was kindly donated by Fisons, salbutamol by Allen and Hanburys, chlorpromazine by May and Baker and ICI 74917 by ICI.

References

BUTCHERS, P.R., FULLARTON, J.R., SKIDMORE, I.K., THOMPSON, L.E., VARDEY, C.J. & WHEELDON, A. (1979). A comparison of the anti-anaphylactic activities of salbutamol and disodium cromoglycate in the rat, the rat mast cell and in human lung tissue. *Br. J. Pharmac.*, **67**, 23–32.

CHURCH, M.K. & GRADIDGE, C.F. (1978). Potentiation of histamine release by sodium cromoglycate. *Life Sci.*, **23**, 1899–1904.

ORR, T.S.C., HALL, D.E., GWILLIAM, J.M. & COX, J.S.G. (1971). The effect of disodium cromoglycate on the release of histamine and degranulation of rat mast cells induced by compound 48/80. *Life Sci.*, **10**, 805–812.

SUNG, C-P., SAUNDERS, J.L., LENHARDT, E. & CHAKRIN, L.W. (1977). Further studies on the tachyphylaxis to DSCG; the effects of concentration and temperature. *Int. Archs Allergy appl. Immun.*, **55**, 385–394.

Effect of halothane on colchicine binding by tubulin

G.A. VERGARA & A. LIVINGSTON

Department of Pharmacology, Medical School, Bristol BS8 1TD

Microtubules are important in many cell processes, the sub-unit of microtubules is tubulin, a protein of 120,000 Daltons, whose polymerization is temperature dependent and can be affected by many drugs, such as colchicine which binds to the sub-unit and hence inhibits polymerization. It has been reported that halothane has some effect on polymerization of tubulin (Hinkley, 1976; Livingston & Vergara, 1979) and we have examined the effects of halothane on the binding of colchicine to tubulin. Whole brains from male rats were used, homogenised with buffer PIPES (0.1 M) EGTA (1 mM), GTP (0.1 mM), centrifuged and then submitted to purification mainly according to Borisy, Olmsted, Marcum & Allen (1974), consisting in incubation and then centrifugation, both at 37°C and then incubation and centrifugation at 0°C. The last supernatant (purified tubulin) was incubated with [^{3}H]-colchicine in the presence (20 µl in 0.5 ml of supernatant) and absence of halothane and the colchicine bound to the tubulin was separated from the free colchicine by gel filtration using a column of 11 cm in height by 0.8 cm in diameter of Ultrogel AcA 54 (fractionation range 5,000–70,000 Daltons). Figure 1 shows the amount of [^{3}H]-colchicine bound to tubulin (in mmoles/mole) against the concentration of colchi-

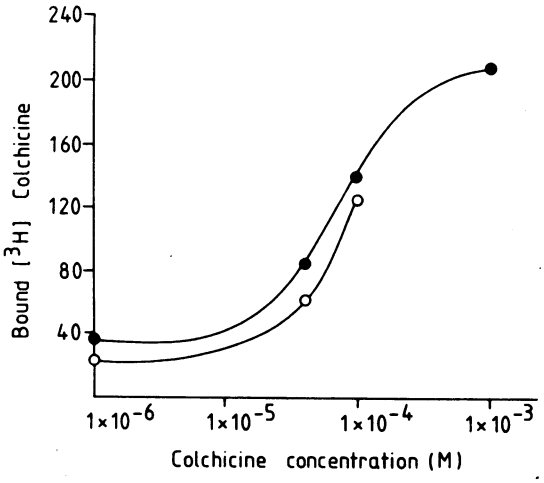


Figure 1 The bound [^{3}H]-colchicine in milli moles per mole of tubulin for different concentrations of colchicine. ● supernatant incubated with [^{3}H]-colchicine ○ supernatant incubated with [^{3}H]-colchicine + 20 µl halothane.

cine present. Both curves appear sigmoid in shape and when halothane was present the amount of bound colchicine was reduced which indicated some interference by halothane in the affinity of colchicine for the binding site on the tubulin molecule. This would indicate that it is possible for halothane to interfere with the polymerization of tubulin into microtubules, this finding *in vitro* would support morphological observations in incubated tissue (Livingston & Vergara, 1979).

This work was supported by the M.R.C.

References

BORISY, G.G., OLMSTED, J.B., MARCUM, J.M. & ALLEN, C. (1974). Microtubule assembly in vitro. *Federation Proc.*, **33**, 167–174.

HINKLEY, R.E. (1976). Microtubule-macrotubule transformations induced by volatile anaesthetics. *J. Ultrastr. Res.*, **57**, 237–250.

LIVINGSTON, A. & VERGARA, G.A. (1979). Effect of halothane on microtubule number in unmyelinated axons from rat sciatic nerve. *Brit. J. Pharm.*, **67**, 473P.

Intraocular pressure and microtubule inhibitors: The iris-ciliary processes as a possible site of action

P. BHATTACHERJEE & R.N. WILLIAMS

Department of Pharmacology, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS

Recently Bhattacherjee & Eakins (1978) reported the lowering of rabbit intraocular pressure (IOP) by colchicine and vinblastine and tentatively suggested that disruption of microtubule assembly by these drugs may be responsible for producing such an ocular response.

In this study we have examined the effects of various microtubule inhibitors on rabbit IOP after subacute topical administration or intravitreal injection. Lumicolchicine, an isomer of colchicine which does not disrupt microtubules (McClure & Paulson, 1977) was also investigated. In addition the distribution of tritiated colchicine and vinblastine was studied in a number of ocular tissues and the binding of these drugs in subcellular fractions of the iris-ciliary processes examined.

With the exception of lumicolchicine and topical demecolcine all the drugs induced a fall in IOP (Table 1) which lasted for 3 days or more after intravitreal injection. After 2–4 days of topical treatment (once/24 h) tolerance to the IOP response developed and by 9–14 days the drugs had no effect unless the dose was increased. Interestingly, there was no cross tolerance between colchicine and vinblastine.

A comparison of the distribution of colchicine in ocular tissues after topical administration and intravitreal injection showed that only the iris-ciliary processes had appreciable levels present, especially after the latter route of administration. Further, most of the tritiated colchicine and vinblastine, 24 h after intravitreal injection was associated with the 100,000 *g* soluble fraction and by using a method modified from Wilson (1975) we found that these drugs were bound (colchicine 15.3% ± 3.9, vinblastine 62.2% ± 25.7) within this fraction, presumably to tubulin.

Since the steady-state IOP depends on the dynamic equilibrium between the rate of formation of aqueous humour by the iris-ciliary processes and its drainage through the anterior chamber angle and scleral vessels, alterations in any one of these regulatory mechanisms would affect IOP.

Accumulation of substantial amounts of microtubule inhibitors by the iris-ciliary processes and the fact that small doses of colchicine and vinblastine administered intraocularly are much more effective in lowering IOP, suggest that decreased formation of aqueous humor by the ciliary processes, resulting

Table 1 Intra-ocular pressure responses produced by microtubule inhibitors 24 h after topical administration or intravitreal injection

Microtubule Inhibitor	*Topical Dose (μg)*	*n*	*Change in IOP Test-Control Eye (mm Hg)*	*Intravitreal Dose (μg)*	*n*	*Change in IOP Test-Control Eye (mm Hg)*
Colchicine	20	7	−5.14 ± 1.05	20	2	−8.5 ± 2.5
	10	4	−5.0 ± 0.0	10	2	−7.25 ± 3.5
	5	5	−2.2 ± 0.58	0.5	4	−2.3 ± 0.85
Vinblastine	5	5	−4.3 ± 0.49	5	2	−10.0 ± 0.5
	2	5	−2.4 ± 0.66	2.5	2	−6.24 ± 2.8
	1	5	−1.8 ± 0.58			
Podophyllotoxin	20	3	−2.8 ± 0.88	5	3	−3.75 ± 0.75
Demecolcine	20	3	0.0	10	3	−5.66 ± 0.33

from microtubule disruption, may be responsible for ocular hypotension.

References

BHATTACHERJEE, P. & EAKINS, K.E. (1978). The intraocular pressure lowering effect of colchicine. *Exp. Eye Res.*, **27**, 649–653.

WILSON, L. (1975). Microtubes as drug receptors: Pharmacological properties of microtubulin protein. *Ann. N.Y. Acad. Sci.*, **253**, 213–231.

MCCLURE, W.O. & PAULSON, J.C. (1977). The interaction of colchicine and some related alkaloids with rat brain tubulin. *Molecular Pharmacology*, **13**, 560–575.

A method for the analysis of drug-receptor interactions on platelets in human whole blood *in vitro*

P.P.A. HUMPHREY & P. LUMLEY

Department of Pharmacology, Glaxo Group Research Limited, Ware Division, Ware, Herts.

Many agents induce platelet aggregation by interaction with specific receptors (Drummond, 1976). However, agonist-antagonist interactions on platelet receptors have rarely, if ever, been studied using classical pharmacological approaches (cf Arunlakshana & Schild, 1959). Hence characterization of these receptors has been hampered. We are now using a new electronic platelet counting technique to characterize some of these receptors on human platelets in whole blood *in vitro*.

The counting technique used has been described elsewhere (Butchers, Humphrey, Hyde, Lumley & Spurling, 1980). Briefly, aliquots (0.5 ml) of fresh human blood (containing 12.9×10^{-3} mol/l trisodium citrate) were incubated with shaking at 37°C. Platelet numbers were counted using the Ultra-Flo 100 platelet counter. Aspirin (2.0×10^{-3} mol/l) was included in all experiments to prevent effects mediated via the release of endogenous cyclooxygenase products (Flower, 1974). The number of platelets in each aliquot was counted before (control) and 1, 2, 3, 4, 5, 10, 20 and 30 min after the addition of an aggregating agent. The platelet number decreased in response to adenosine diphosphate (ADP, 3×10^{-7}–10^{-5} mol/l), adrenaline (3×10^{-7}–10^{-5} mol/l), U-46619 ($3 \times 10^{-9} - 3 \times 10^{-7}$ mol/l) and collagen (0.1–40 μg/ml) with peak decreases at 1–2 min, 5 min, 5 min and 5–10 min respectively. Concentration-effect curves were obtained by plotting the lowest platelet number counted for each concentration of aggregating agent studied, as a percentage of the control platelet number, against concentration.

ADP, U-46619 and collagen produced up to a 98% reduction in platelet numbers in a concentration-dependent manner with a 50% reduction in platelet numbers at concentrations (PC_{50} values) of 1.1 (0.8–1.5) $\times 10^{-6}$ mol/l, 4.7 (1.3–17.5) $\times 10^{-8}$ mol/l and 3.5 (2.0–5.9) μg/ml respectively (geometric mean, 95% confidence limits, $n = 4$ for each agonist). Adrenaline produced a maximum decrease in platelet numbers of about 70% with a PC_{50} value of 5.6 (2.1.–15.5) $\times 10^{-7}$ mol/l ($n = 4$). The effects of these agonists, with the exception of high concentrations of

collagen (40 μg/ml), were abolished by pre-incubation of the blood with ethylenediaminetetra-acetic acid (3.0×10^{-3} mol/l).

When the blood was incubated with phentolamine (1.0×10^{-7}, 1.0×10^{-6}, or 1.0×10^{-5} mol/l) for 10 min, adrenaline concentration-effect curves were displaced to the right in a parallel manner such that the PC_{50} values for adrenaline increased with increasing concentrations of phentolamine. Analysis of this data gave a mean pA_2 value (95% confidence limits, $n = 6$) of 7.38 (7.12–7.63) and a slope of 0.99 (0.84–1.15). The highest concentration of phentolamine (1.0×10^{-5} mol/l) used had no effect on ADP concentration-effect curves. Our pA_2 value in platelets is close to the pA_2 value for phentolamine of 7.76 on α-adrenoceptors in the human isolated digital artery (Jauernig & Moulds, 1978). These results suggest that the method offers a reproducible and reliable way of studying agonist–antagonist interactions on human platelets in whole blood *in vitro*.

We are grateful to Karen Bunyan and Rosemary Farrington for skilled technical assistance.

References

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

BUTCHERS, JANET, HUMPHREY, P.P.A., HYDE, J.J., LUMLEY, P. & SPURLING, N.W. (1980). The evaluation of a new electronic counting technique for measurement of platelet aggregation in human whole blood *in vitro*. *Br. J. Pharmac.* **70**, 160P–161P.

DRUMMOND, A.H. (1976). Interactions of blood platelets with biogenic amines: uptake, stimulation and receptor binding. In: *Platelets in Biology and Pathology*. Ed. Gordon, J.L. pp 203–239. Amsterdam: Elsevier.

FLOWER, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis *Pharmac. revs.*, **26**, 33–67.

JAUERNIG, R.A. & MOULDS, F.W. (1978). A human arterial preparation for studying the effects of vasoactive agents. *Circ. Res.*, **42**, 363–368.

Interaction of phenytoin with antacid constituents and kaolin

J.C. McELNAY (introduced by P.F. D'ARCY)

Department of Pharmacy, Medical Biology Centre, The Queen's University of Belfast, 97 Lisburn Road, Belfast, BT9 7BL, Northern Ireland

It has been suggested that antacids may alter the pharmacokinetic profile of phenytoin (Kutt, 1975) and indeed the work of Kulshrestha *et al.* (1978) indicated that a magnesium trisilicate/aluminium hydroxide gel mixture, when administered in therapeutic dosage with phenytoin, caused a significant fall in phenytoin plasma levels. This finding, however, was not supported by the work of Chapron *et al.* (1979). Dimethicones are being increasingly used as antiflatulent agents in proprietary antacid preparations and it therefore seemed important to investigate the effects of dimethicone in relation to other antacid constituents on the absorption of phenytoin.

The work was carried out *in vitro* using an everted rat intestinal model. The method consisted of separately collecting drug absorbed across two consecutive intestine segments from the same rat. The segments were bathed in separate buffer filled tissue chambers. The control and test chambers contained phenytoin sodium (36 mg) while the test chamber also contained a clinically equivalent dose of antacid constituent. Cumulative phenytoin transferred across the segments was followed for 100 minutes. Each constituent was tested in triplicate and any changes noted in phenytoin absorption are shown in table 1. The results show that dimethicone and kaolin gave large decreases in phenytoin absorption while an increase was

Table 1 Effects of antacid constituents on the absorption of phenytoin

Antacid constituent	*Amount of constituent used*	*Change in total phenytoin absorbed (%)*
Dimethicone (activated)	150 mg	−71.3
Bismuth carbonate	250 mg	+28.2
Magnesium trisilicate	250 mg	−6.2
Light kaolin (an adsorbant)	1 gm	−60.2

seen in the case of bismuth carbonate. Magnesium trisilicate did not noticeably affect the absorption. The results of the present study therefore indicate that certain antacid constituents may give rise to changes in phenytoin absorption kinetics and/or bioavailability. Patients stabilised on phenytoin therapy should perhaps be made aware of this potential interaction mechanism, especially with regard to freely available 'over the counter preparations'.

References

CHAPRON, D.J., KRAMER, P.A., MARIANO, S.A. & HOHNADEL, A.C. (1979). Effect of calcium and antacids on phenytoin bioavailability. *Arch. Neurol.*, **36**, 436–438.

KULSHRESTHA, V. K., THOMAS, M., WADSWORTH, J. & RICHENS, A. (1978). Interaction between phenytoin and antacids. *Br. J. clin. Pharmacol.*, **6**, 177–179.

KUTT, H. (1975). Interactions of antiepileptic drugs. *Epilepsia*, **16**, 393–402.

Use of rat isolated renal tubules for evaluation of the mechanisms of aminoglycoside nephrotoxicity

S.B. CHAHWALA & E.S. HARPUR

Pharmacology Laboratories, Department of Pharmacy, University of Aston in Birmingham, Birmingham B4 7ET

The aminoglycoside antibiotics, e.g. gentamicin, are known to be ototoxic and nephrotoxic. Urinary levels of lysosomal enzymes such as acid phosphatase (AP) and N-acetyl-β-glucosaminidase (NAG) appear to be a sensitive index of proximal tubular damage. Autoradiographic studies have indicated that gentamicin is transported into the proximal tubular cells by endocytosis and later sequestered in the lysosomes (Just, Erdmann & Habermann, 1977).

Male wistar rats (150–200 g) were killed and the excised kidney cortices were pooled and digested with collagenase. The further preparation of the tubules was adapted from the method of MacDonald & Saggerson, (1977). The tubules were incubated with shaking at 37°C in Krebs-Henseleit bicarbonate buffer (pH 7.4) containing pyruvate (5 mM). Each 25 ml flask, containing an incubation volume of 4 ml, was continuously gassed with a mixture of 95% O_2 and 5% CO_2.

The morphology and viability of the cells was ascertained by phase contrast and electron microscopy and also by gluconeogenesis and uptake of [^{14}C]-lysine. Glucose production was linear for 60 min incubation (237 $\pm$ 14 nmoles glucose/mg protein at 60 min: $n = 3$) at which time the tissue:medium distribution ratio of lysine was >8.

The effect of gentamicin on the release of AP and NAG from isolated tubules was investigated over a 2.5 h incubation period. At 60 min gentamicin (10^{-3} M) maximally inhibited release of both enzymes (AP = 67% and NAG = 71% of control enzyme activity; $n = 3$). On further incubation this effect was reversed and at 150 min the enzyme release in the presence of the drug was AP = 162% and NAG = 117% of the control value. The dependence of AP and NAG release on the concentration of gentamicin in the incubation medium was also investigated. After 60 min incubation low concentrations of gentamicin (10^{-7}–10^{-6} M) increased the release of enzymes over the control but this effect was reversed at higher concentrations and the release was maximally inhibited in the range 5×10^{-4} M–10^{-3} M.

The influence of gentamicin on lysosomal enzyme release was found to be a function of the gentamicin: protein concentration ratio. Whereas, after 60 min incubation, gentamicin (10^{-3} M) caused inhibition of release of AP (71%) and NAG (61%) at a protein concentration of 0.2 mg/ml, the effect was abolished at higher protein concentrations (0.6–0.8 mg/ml).

Interpretation of these findings may be possible when the results of our studies of the kinetics of gentamicin uptake into renal tubules become available.

References

JUST, M., ERDMANN, G. & HABERMANN, E. (1977). The renal handling of Polybasic drugs. 1. Gentamicin and aprotinin in intact animals. *Naunyn-Schmiedeberg's Arch. Pharmac.*, **300**, 57–66.

MACDONALD, D.W. & SAGGERSON, E.D. (1977). Hormonal control of glyconeogenesis in tubule fragments from renal cortex of fed rats. *Biochem. J.*, **168**, 33–42.

Responses of rabbit isolated basilar artery to transmural nerve stimulation, noradrenaline and 5-hydroxytryptamine

N. ANWAR & D.F.J. MASON

Department of Pharmacology, St. Bartholomew's Medical College, London E.C.1M 6BQ

Histo-fluorescence studies show that cerebral vessels are richly supplied by adrenergic neurones but the neuro-effector mechanisms may be unconventional (Heistad & Marcus, 1978). Responses to field stimulation of rabbit basilar artery are abolished by chronic superior cervical ganglionectomy but are not reduced by α-receptor antagonists—phenoxybenzamine and phentolamine (Lee *et al*, 1976). The α-adrenoceptors in cerebral vessels are relatively insensitive to noradrenaline and exhibit a very high dissociation constant for phentolamine (Duckles & Bevan, 1976). Isoprenaline produces an α-receptor mediated vasoconstriction and phenylephrine is a less potent vasoconstrictor than isoprenaline in cat middle cerebral artery (Edvinson & Owman, 1974). We have compared the responses of rabbit basilar artery to transmural stimulation, noradrenaline and 5-hydroxytryptamine.

Ring segments (5 mm long) of the artery were mounted on stainless steel wires and suspended under a resting tension of 0.5 *g* in a 20 ml bath with oxygenated Krebs solution at 37°C. Changes in tension were recorded with a force displacement transducer. Transmural stimulation (1 ms, 20 V 10–20 Hz for 15 s) was carried out with a pair of stimulating electrodes placed one on either side of the vessel. Drugs were added directly to the bath. Stimulation produced a frequency dependent constrictor response in 12 out of 15 preparations, but in three others a dilator response was seen. The constrictor response consisted of initial rapid and ensuing tonic phases, both of which were blocked by tetrodotoxin (3×10^{-7} M). The maximal response (at 20 Hz and above) was 0.32 ± 0.09 g, compared to the maximum to noradrenaline which was 1.12 ± 0.26 g. The constrictor response remained unaffected in the presence of the uptake -1 inhibitor, cocaine (10^{-6}–10^{-5} M), the adrenergic neurone blocker, guanethidine (10^{-6}–10^{-5} M), the β-receptor blocking agent, propranolol (10^{-6} M), the α-receptor blocking agents—phentolamine (10^{-8}–10^{-6} M) and phenoxybenzamine (10^{-8}–10^{-6} M) and the 5-HT-receptor blocking agent, methysergide (10^{-7}–10^{-5} M). In reserpinised animals (3 mg/Kg over 48 h), the constrictor response was replaced by a dilator response which was unaffected by propranolol (10^{-6} M), atropine (10^{-7} M) and mepyramine (10^{-6} M) but abolished by tetrodotoxin. Similar results were obtained with the 3 preparations from normal animals which showed a dilator response. The vessel was much more sensitive to added 5-hydroxytryptamine than to noradrenaline (ED_{50} for 5-HT 2.4×10^{-8} M and for NA 8.4×10^{-6} M). The responses to noradrenaline were antagonized competitively by phentolamine (pA_2 against NA—6.8), while responses to 5-HT were not influenced. However, methysergide (3×10^{-8}–3×10^{-6} M) shifted the 5-HT dose-response curve to the right with a reduction in maximal response. Responses to noradrenaline were not influenced.

The results are in keeping with the suggestion that α-adrenoceptors in cerebral vessels are atypical and relatively insensitive to noradrenaline, which might account for the smaller maximal response with neural stimulation. The demonstration of a dilator response to field stimuli in reserpinised animals and failure of α-adrenoceptor blocking agents to block constrictor and dilator responses to stimulation raise the possibility that noradrenaline may not be the only transmitter involved.

References

DUCKLES S.P. & BEVAN J.A. (1976). Pharmacological characterization of adrenergic receptors of a rabbit cerebral artery *in vitro*. *J. Pharm. exp. Ther.*, **197**, 371–377.

EDVINSON, L. & OWMAN C. (1974). Pharmacological characterization of adrenergic α and β receptors mediating vasometer responses of cerebral arteries *in vitro*. *Circ. Res.*, **35**, 835–849.

HEISTAD, D.D. & MARCUS, M.L. (1978). Evidence that neural mechanisms do not have important effects on cerebral blood flow. *Circ. Res.*, **42**, 295–302.

LEE, T.J.F., SU, C. & BEVAN, J.A. (1976). Neurogenic sympathetic vasoconstriction of the rabbit Basilar artery. *Circ. Res.* **39**, 120–126.

The following communication was presented to the Leeds meeting of the British Pharmacological Society in September, 1979.

Inhibition of [^{3}H]-dexetimide binding by a homologous series of methylfurthrethonium analogues at the peripheral muscarinic receptor

A.J. BELD & J.F. RODRIGUES DE MIRANDA

Pharmacological Institute, University of Nijmegen, Nijmegen, The Netherlands

From binding studies in which the displacement of radiolabelled cholinoceptor antagonists by non-labelled agonists is investigated, it appears that the shape of the inhibition curve deviates substantially from the normal sigmoid form. No such deviations are observed in the inhibition of radio-labelled antagonists by non-labelled antagonists (Birdsall, Burgen & Hulme, 1978).

To demonstrate that the deviation in shape is inherent to the nature of agonism itself and not a consequence of the fact that the agonists and antagonists used in these investigations belong to classes of structurally different compounds, a homologous series of methylfurthrethonium analogues was investigated. In this series there is a gradual change of full agonists via partial agonists to antagonists with increasing chain length of one of the alkyl groups on the quaternary nitrogen (Table 1).

[^{3}H]-Dexetimide binding to a crude plasma membrane fraction of bovine tracheal smooth muscle was inhibited by methylfurthrethonium and its analogues in a concentration dependent manner. Bound radioactivity was measured by rapidly centrifuging the membrane fragments using a microfuge. Affinities and capacities were calculated using a computerized curve fitting procedure.

The results can be explained by assuming that there are two independent binding sites (Ariëns, Beld, Rodrigues de Miranda & Simonis, 1979). One of them has a high affinity for methylfurthrethonium itself, an intermediate affinity for the ethyl, propyl and allyl analogue and a low affinity for the isopropyl and butyl analogue of methylfurthrethonium. The other binding site has a low and almost identical affinity for all ligands studied.

Although our data point to the high affinity binding site as the one most likely to be related to the functional muscarinic receptor, it is not conceivable that the low affinity binding site has no relationship to the receptor at all.

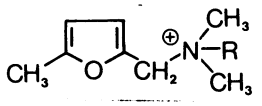
References

BIRDSALL, N.J.M., BURGEN, A.S.V. & HULME, F.C. (1978). The binding of agonists to brain muscarinic receptors. *Mol. Pharm.* **14**, 723–736.

ARIËNS, E.J., BELD, A.J., RODRIGUES DE MIRANDA, J.F. & SIMONIS, A.M., The Receptors. A comprehensive treatise. Ed. R.D. O'Brien, Plenum Press (1979).

VAN ROSSUM, J.M. & HURKMANS, J.A.Th.M. (1962). Molecular pharmacology and enzymology. The dualistic behavior of substrates and stimulant drugs. *Acta Physiol. Pharmacol. Neerlandica* **11**, 173–194.

Table 1



			Model 0204		
R	α*	pD_2/pA_2^*	$-\log K_1$	K_2/K_1	n_H
$-CH_3$	1.0	7.2	6.6	43	0.41
$-CH_2-CH_3$	1.0	6.3	6.5	18	0.58
$-CH_2-CH=CH_2$	—	—	5.7	9.8	0.72
$-CH_2-CH_2-CH_3$	0.9	5.3	5.5	6.8	0.80
$-CH(CH_3)_2$	0	5.8	5.1	1.0	1.0
$-CH_2-CH_2-CH_2-CH_3$	0	4.4	5.2	1.0	1.0

* The *in vivo* data were taken from van Rossum (van Rossum & Hurkmans, 1962). α = intrinsic activity.