Regional variations in [^{3}H]-prazosin and [^{3}H]-noradrenaline binding in the rat brain

R.M. BREMNER & P.M. GREENGRASS
(introduced by M.J. DAVEY)

Department of Medicinal Biology, Pfizer Central Research, Pfizer Limited, Sandwich, Kent

In the peripheral sympathetic nervous system there is considerable evidence for the presence of at least two types of α-adrenoceptor which have been classified as α_1 and α_2. A pre-junctional receptor which inhibits depolarization-induced release of noradrenaline (α_2) and a post-junctional excitatory receptor on vascular smooth muscle (α_1). In addition there is also some evidence for post-junctional α_2 receptors. The limited pharmacological and biochemical evidence available suggests that central α-adrenoceptors may also be subdivided.

Radio-ligand binding techniques are particularly suitable for direct biochemical identification of a variety of central neurotransmitter receptors and several tritiated ligands are available for identification of α-adrenoceptors. The high selectivity of prazosin for peripheral α_1 receptors (Cambridge, Davey & Massingham, 1977) suggested that it would be a suitable ligand for location and identification of these receptors in the central nervous system.

We have compared the distribution of binding of [^{3}H]-prazosin and [^{3}H]-(–) noradrenaline in the rat central nervous system. Freshly dissected tissue from male rats was homogenized in 20 vol (w/v) ice-cold Tris-HCl buffer (pH 7.7 at 25°C), centrifuged (50,000 × g, 10 mins) and washed once by resuspension and recentrifugation. The final resuspension was in 80 vol (w/v) Tris-HCl buffer (pH 8 at 25°C) for [^{3}H]-prazosin and in 40 vol (w/v) Tris-HCl buffer (pH 8 at 25°C) for [^{3}H]-noradrenaline. Aliquots of these tissue suspensions were incubated with 0.2 nM [^{3}H]-prazosin (33 Ci/mmole) at 25°C for 30 minutes. The noradrenaline assay was that described by U'Prichard & Snyder, 1977; homogenates being incubated with 2 nM [^{3}H]-noradrenaline (27.5 Ci/mmole), 1 mM pyrocatechol, 0.1 mMEDTA, 0.01 mMDTT and 0.001% ascorbate at 25°C for 40 minutes. Specific binding was defined as that binding displaced by 1000 nM phentolamine for [^{3}H[-prazosin (90% of total) and 100 nM oxymetazoline for [^{3}H]-noradrenaline (60% of total).

The specific binding was saturable and of high affinity with both ligands. The apparent dissociation constants were similar in all brain areas studied (0.29 nM, [^{3}H]-prazosin, 12 nM [^{3}H]-noradrenaline) although there were differences in the number of binding sites. The density of prazosin binding sites was frontal cortex > hypothalamic-pre-optic area > medulla > pons > striatum > cerebellum > basal hypothalamus. The highest [^{3}H]-noradrenaline binding was in the basal hypothalamus, otherwise the distribution was similar to that for [^{3}H]-prazosin. Studies with α-adrenoceptor blocking drugs suggest that central [^{3}H]-prazosin binding sites closely resemble the α_1 receptor in the peripheral vasculature. Phenoxybenzamine (Ki 1.7 nM) WB 4101 (Ki 1.1 nM) and indoramin (Ki 8 nM) which have greater affinity for α_1 than α_2 receptors, are more potent than piperoxan (Ki 340 nM) and yohimbine (Ki 1 μM) in competing for [^{3}H]-prazosin binding sites in all the brain areas studied. In the case of [^{3}H]-(–)-noradrenaline binding, piperoxan (Ki 100 nM) and yohimbine (Ki 290 nM) are considerably more potent than prazosin (Ki 6 μM) and indoramin (Ki 33 μM). Under these assay conditions [^{3}H]-noradrenaline labels an α-adrenoceptor resembling the peripheral α_2 receptor. Thus [^{3}H]-prazosin and [^{3}H]-noradrenaline appear to label different sub-classes of α-adrenoceptor in the central nervous system. In view of this it is proposed that these ligands may be of value in elucidating the precise role of the different classes of α-adrenoceptor in the central control of many physiological functions including vasomotor tone.

We thank T. Elliot for technical assistance.

References

CAMBRIDGE, D., DAVEY, M.J. & MASSINGHAM, R. (1977). The pharmacology of antihypertensive drugs with special reference to vasodilators, α-adrenergic blocking agents and prazosin. *Med. J. Aust.* Specl. Suppl., **2**, 2.

U'PRICHARD, D.C. & SNYDER, S.H. (1977). Binding of ^{3}H-catecholamines to α-noradrenergic receptor sites in calf brain. *J. Biol. Chem.*, **252**, 6450.

Some characteristics of the gastric antisecretory actions of prostaglandins in mammalian perfused whole-stomachs *in vitro*

N.K. BOUGHTON-SMITH &
B.J.R. WHITTLE

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

The recent development of viable, acid-secreting preparations of mammalian stomachs (Holton & Spencer, 1976; Bunce & Parsons, 1976; Main & Pearce, 1978; Angus, Black & Stone, 1978) has allowed study of the antisecretory actions of drugs, including prostaglandins, without such complicating factors as mucosal blood flow changes. In both the isolated stripped mucosa and lumen-perfused whole stomach of the rat, prostaglandin E_2 has been shown to inhibit histamine-stimulated acid output (Main &

Pearce, 1978; Whittle, Boughton-Smith, Moncada & Vane, 1978). We have now investigated in more detail the characteristics of the responses to antisecretory prostaglandins during different rates of histamine stimulation in both rat and mouse whole stomach.

Whole stomachs from mice or immature rats were removed to a 20 ml organ bath as described before (Bunce & Parsons, 1976; Angus, Black & Stone, 1978). The serosal solution contained (mM) NaCl 119, KCl 4.7, $MgSO_4$ 1.2, glucose 5.6, $NaHCO_3$ 30, KH_2PO_4 0.5 and $CaCl_2$ 1.0 (pH 7.6) and was gassed with 95% O_2, 5% CO_2. The mucosal solution, with buffer salts omitted and gassed with O_2, was perfused (1 ml/min) through the gastric lumen and the acid output was detected by changes in pH via a microelectrode system. In most experiments, prostaglandins were added to the serosal bathing solution and the preparation incubated for 12 min prior to addition of the secretagogue, histamine, which was also added to the serosal solution.

In the rat stomach, histamine (1.25–20 μg/ml; 0.4–7 × 10^{-5}M) caused a dose-dependent rise in the resting acid secretion (13.2 ± 0.6 μmol × 10^{-2}/min, mean ± s.e. mean, n = 106) reaching a maximal response of 22.1 ± 1.6 μmol × 10^{-2}/min (n = 37) over basal with the dose of histamine (20 μg/ml). Pre-incubation with PGE_2 (0.1–1 μg/ml; 0.3–3 × 10^{-6}M) caused a dose-related parallel shift to the right of the dose-response relationship for histamine. At maximally-stimulated acid-output with histamine (20 μg/ml), the inhibition by PGE_2 (1 μg/ml) was 53.5 ± 7.1% (n = 27; P<0.001). This inhibition was surmountable; with supra-maximal concentrations of histamine (40–80 μg/ml) there was no longer any significant (P>0.05) inhibition of acid output by PGE_2 (1 μg/ml). A parallel shift of the dose-response curve to histamine was also observed with the prostacyclin analogue, 6β-PGI, as will be demonstrated.

In the mouse whole stomach, histamine (1.25–20 μg/ml) likewise caused a dose-dependent rise in acid output with the maximal response being obtained with comparable doses to those in the rat. The mouse stomach had a resting acid output of 11.9 ± 0.8 μmol 10^{-2}/min; n = 84 and a maximal acid output of 29.2 ± 4.8 μmol × 10^{-2}/min over basal; n = 10. In contrast to the rat, no significant inhibition of acid output could be obtained by pre-incubation with PGE_2 (1–5 μg/ml) even at low rates of histamine (1.25 μg/ml)-stimulation. Whether the lack of antisecretory action with PGE_2 in the mouse stomach *in vitro*, in doses having marked antisecretory activity in the rat stomach is the consequence of rapid metabolism or failure of the prostaglandin to reach its site of action requires further evaluation. These results contrast with the potent antisecretory activity of histamine H_2-receptor antagonists in the mouse stomach (Angus, Black & Stone, 1978).

The present study reveals that in the isolated rat stomach, the degree of inhibition of acid secretion by prostaglandins in the concentrations used, is both related to the level of stimulation and is surmountable. Care must therefore be taken when comparing the antisecretory actions of prostaglandins against different secretagogues *in vitro* especially if different rates of acid secretion are obtained. The mechanisms underlying these characteristics of inhibition are as yet obscure. However, since PGE_2 and prostacyclin analogues may act by reducing parietal-cell cyclic AMP concentrations (Soll & Whittle, 1979), the nature of the interaction at the level of the adenylate cyclase may warrant analysis.

We thank G. Steel for technical assistance.

References

ANGUS, J.A., BLACK, J.W. & STONE, M. (1978). Comparative assay of histamine H_2-receptor antagonists using the isolated mouse stomach. *Br. J. Pharmac.*, **62**, 445–446P.

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

HOLTON, P. & SPENCER, J. (1976). Acid secretion by guinea-pig isolated stomach. *J. Physiol.* (Lond.), **255**, 465–479.

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

SOLL, A.H. & WHITTLE, B.J.R. (1979). Activity of prostacyclin, a stable analogue 6β-PGI_1 and 6-oxo-$PGF_{1\alpha}$ on canine isolated parietal cells. *Br. J. Pharmac.*, (this meeting).

WHITTLE, B.J.R., BOUGHTON-SMITH, N.K., MONCADA, S. & VANE, J.R. (1978). Activity of prostacyclin (PGI_2) and its product 6-oxo-$PGF_{1\alpha}$ on the rat gastric mucosa *in vivo* and *in vitro*. *Prostaglandins*, **15**, 955–968.

The action of salmon calcitonin on indomethacin-induced gastric ulceration in the mouse

R.F.L. BATES, G.A. BUCKLEY & R.J. STRETTLE

Department of Life Sciences, Trent Polytechnic, Burton Street, Nottingham

Many of the non-steroidal antiinflammatory drugs, such as indomethacin and aspirin, may induce gastric ulceration. In contrast the hormone calcitonin has an antiinflammatory action in several animal models (Abdullahi, De Bastiani, Nogarin & Velo, 1975) but inhibits gastric acid secretion (Becker, Konturek, Reeder & Thompson, 1973) and gastric ulceration induced by stress (Barlet, 1974) or histamine (Barlet & Bates, 1974). These latter inhibitory effects of