References

APPERLEY, EIRA, HUMPHREY, P.P.A. & LEVY, G.P. (1977). Two types of excitatory receptor for 5-hydroxytryptamine in dog vasculature? *Br. J. Pharmac.*, **61**, 465P.

FARNEBO, L.O. & HAMBERGER, B. (1974). Regulation of [^{3}H]-5-hydroxytryptamine release from rat brain slices. *J. Pharm. Pharmac.*, **26**, 642–643.

HAIGLER, H.J. & AGHAJANIAN, G.K. (1977). Serotonin receptors in the brain. *Fed. Proc.*, **36**, 2159–2164.

McGRATH, M.A. & SHEPHERD, J.T. (1976). Inhibition of adrenergic neurotransmission in vascular smooth muscle by 5-hydroxytryptamine. *Circulation*, **53** and **54**, Suppl. II–20, 0071.

PAGE, I.H. & McCUBBIN, J.W. (1953). The variable arterial blood pressure response to serotonin in laboratory animals and man. *Circ. Res.*, **1**, 354–362.

VANHOUTTE, P.M. & SHEPHERD, J.T. (1973). Venous relaxation caused by acetylcholine acting on sympathetic nerves. *Circ. Res.*, **32**, 259–267.

VERHAEGHE, R.H., VANHOUTTE, P.M. & SHEPHERD, J.T. (1977). Inhibition of sympathetic neurotransmission in canine blood vessels by adenosine and adenine nucleotides. *Circ. Res.*, **40**, 208–215.

Characterization of the β-adrenoceptors in the mesovarium of the rat

G.H. APPERLEY, R.T. BRITTAIN, R.A. COLEMAN, I. KENNEDY & G.P. LEVY

Department of Pharmacology, Allen & Hanburys Research Ltd., Ware, Hertfordshire SG12 0DJ, England.

The mesovarium is the peritoneal fold which holds the ovary in place. In the rat the mesovarium consists of connective tissue, adipose tissue and smooth muscle. Nelson & Kelly (1971) have reported that prolonged oral administration of the selective β_2-stimulant soterenol, 4.6–21.5 mg/kg daily for 18 months, resulted in the formation of benign tumours of the smooth muscle (leiomyomas) in the mesovarium of Sprague–Dawley rats. In this or in a Sprague–Dawley-derived strain of rat, prolonged oral administration of high doses of all other β_2-stimulants examined, including mesuprine (Nelson, Kelly & Weikel, 1972) and salbutamol (Poynter, Harris & Jack, 1978), caused mesovarian leiomyomas. Since mesovarian leiomyomas are normally uncommon, it is possible that the effect is mediated through activation of β-adrenoceptors. Therefore, we have investigated whether the rat mesovarian smooth muscle contains β-adrenoceptors and, if so, which subtype is present.

Isolated mesovarian strips from Charles River CD (Sprague–Dawley derived) rats, 300–400 g, were set up in Krebs solution at 37°C bubbled with 5% CO_2 in O_2. Since the preparation has no spontaneous tone the relaxant effects of β-stimulants were determined in preparations contracted with KCl ($3–6 \times 10^{-2}$ mol/l); phenoxybenzamine (7×10^{-7} mol/l) was present to eliminate actions at α-adrenoceptors. (−)Isoprenaline (0.01–100 ng/ml), (−)adrenaline

Table 1 Relative β-stimulant potencies of (−)isoprenaline, (−)adrenaline, (±)salbutamol and (−)noradrenaline on isolated rat mesovarium and guinea-pig trachea and atria

Preparation and response measured	*Receptor type*	*Mean equipotent concentration (95% confidence limits)* (−)*Isoprenaline*	(−)*Adrenaline*	(±)*Salbutamol*	(−)*Noradrenaline*
Rat mesovarium Relaxation of KCl-induced contraction	β_2	1	5 (3–8)	30 (20–47)	344 (129–914)
Guinea-pig tracheal strip Relaxation of tone	$\beta_2 > \beta_1$	1	9 (8–10)	28 (21–38)	24 (18–30)
Guinea-pig right atria Increase in rate of contraction	β_1	1	31 (16–58)	1222* (552–2707)	13 (8–20)
Guinea-pig left atria Increase in force of contraction	β_1	1	45 (26–79)	>10000*	16 (8–30)

* Partial agonist. All experiments were carried out in presence of phenoxybenzamine (7×10^{-7} mol/l). Each mean equipotent concentration was obtained from not less than 5 individual experiments.

(0.1–100 ng/ml), (±)salbutamol (0.1–100 ng/ml) and (−)noradrenaline (1–10,000 ng/ml) caused concentration-dependent relaxations, the concentration-effect curves being similar in slope and maxima. Relative potencies are summarized in Table 1 which includes comparative data obtained on guinea-pig isolated tracheal and right and left atrial strips.

The rank order of potency of the catecholamines in causing relaxation of the rat mesovarium was isoprenaline > adrenaline ≫ noradrenaline. The potency of (±)salbutamol relative to (−)isoprenaline was similar to that on the guinea-pig isolated trachea; other β_2-stimulants such as (±)terbutaline (0.1–1000 ng/ml) and (±)fenoterol (0.01–100 ng/ml) were also potent agonists on the rat mesovarium. Propranolol was a potent antagonist of (−)isoprenaline-induced relaxations of rat mesovarium (pA_2 = 8.8); in contrast, practolol was only a weak antagonist (pA_2 = 5.0–5.3).

We conclude that rat mesovarian smooth muscle contains β-adrenoceptors and that these are of the β_2-subtype. It is possible therefore that the leiomyomas referred to above result from prolonged and intense activation of β_2-adrenoceptors in mesovarian smooth muscle.

References

NELSON, L.W. & KELLY, W.A. (1971). Mesovarial leiomyomas in rats in a chronic toxicity study of soterenol hydrochloride. *Vet. Path.*, **8**, 452–457.

NELSON, L.W., KELLY, W.A. & WEIKEL, J.H. (1972). Mesovarial leiomyomas in rats in a chronic toxicity study of mesuprine hydrochloride. *Tox. Appl. Pharmac.*, **23**, 731–737.

POYNTER, D., HARRIS, D.M. & JACK, D. (1978). Salbutamol: Lack of oncogenic activity in man. *Br. med. J.* (in press).

Experimental atherosclerosis in the Wistar rat

D.V. PARKE, P.J. SACRA & P.C. THORNTON

Department of Biochemistry, University of Surrey and Department of Pharmacology, Biorex Laboratories Ltd., London.

The search for a laboratory model to study potential anti-atherosclerotic compounds has been well documented (Constantinides, 1965; Kritchevsky, 1974). The rat has received little attention and is generally considered to be resistant to the induction of atheromatous lesions. However, following the reports of Altman (1972) and Testa, Canestrini & Oldani (1975) a short-term induction of atherosclerosis in the rat seemed possible using large doses of vitamin D_2 and cholesterol. We have undertaken a series of experiments to investigate this methodology.

Groups of male Biorex Wistar rats were used and treated orally (Table 1) with vitamin D_2 suspension (Duphasol, Duphar, 1000 iu/mg, 4 ml/kg in distilled water) and cholesterol (BDH, 2 ml/kg in olive oil experiments 1 and 2, 1.2 ml/kg in isopropyl myristate experiment 3). At termination the animals were exsanguinated under ether anaesthesia, serum collected and stored at −21°C for cholesterol assay. The aortas were dissected out from the heart to the bifurcation and weighed. Liver weights were also recorded.

Experiment 1

The higher dose of vitamin D_2 (480,000 iu/kg) and cholesterol (60 mg/kg) produced almost a 3-fold increase in aorta weight and a 57% increase in plasma cholesterol levels but resulted in a mortality rate of 83%. The lower dose regimen over 5 and 7 days produced similar increases but the aorta weights were not significantly different from controls.

Experiment 2

A reduced vitamin D_2 treatment resulted in decreased mortality. Aorta weights and plasma cholesterol levels were increased significantly.

Experiment 3

Reducing the vitamin D_2 treatment to 1 day prevented mortalities, did not alter aorta weights but did increase plasma cholesterol levels ($P < 0.01$).

Toxic doses of vitamin D_2 and high cholesterol intake will produce lipid deposition and hyperlipidaemia in the rat but when this is severe enough to enable treatment for screening purposes undue mortality is encountered, not reported by Altman (1972) and Testa *et al.* (1975), making the method unsuitable for drug evaluation.

References

ALTMAN, R.F.A. (1972). A simple method for the rapid production of atherosclerosis in rats. *Experientia*, **29**, 256.