Br. J. Pharmacol. (1994), **113**, 699–702

Atypical β-adrenoceptors in the rat isolated common carotid artery

Mabayoje Ayotunde Oriowo

Department of Pharmacology & Toxicology, Faculty of Medicine, Kuwait University, P.O. Box 24923, Safat 13110, Kuwait

1 The possible existence of atypical β-adrenoceptors in vascular smooth muscle of the rat common carotid artery was examined in this study.

2 Isoprenaline produced concentration-dependent relaxation of noradrenaline (10^{-7} M) precontracted ring segments of the carotid artery. The relaxation was not affected by endothelial denudation.

3 Propranolol (10^{-8} M–3×10^{-7} M) shifted the isoprenaline curve to the right without suppressing the maximum response. However, the slope (0.74) of the Schild plot was significantly ($P<0.05$) less than 1.

4 Salbutamol (β_2), CGP 12177 and BRL 37344 (β_3) also concentration-dependently relaxed noradrenaline precontracted artery segments. These relaxations were not affected by propranolol (10^{-7} M). Pretreatment of the artery segments with BRL 37344 did not desensitize the tissue to the relaxant effect of isoprenaline, CGP 12177 and salbutamol.

5 It is concluded that atypical β-adrenoceptors exist in vascular smooth muscle of the common carotid artery.

Keywords: Rat common carotid artery; atypical β-adrenoceptors; (±)-CGP 12177; BRL 37344

Introduction

β-Adrenoceptors were classified into β_1- and β_2-adrenoceptors based on the differential affinities of some tissues to a series of sympathomimetic amines by Lands *et al.* (1967a). Since then, there have been reports of responses produced by β-adrenoceptor agonists which are relatively insensitive to propranolol and other β-adrenoceptor antagonists. Such responses include lipolysis in rat adipose tissue (Arch *et al.*, 1984; Wilson *et al.*, 1984), relaxation of rat proximal (Bianchetti & Manara, 1990) and distal colon (McLaughlin & MacDonald, 1990; Kirkham & Kelly, 1992), fundus (McLaughlin & MacDonald, 1991) and guinea-pig ileum (Bond & Clarke, 1988). These atypical β-adrenoceptors are designated β_3-adrenoceptors (Arch, 1989). The distribution of these receptors is not limited to the gastrointestinal tract. Clark & Bertholet (1983) demonstrated a vasorelaxant effect of pindolol, a non-specific β-adrenoceptor antagonist with significant intrinsic sympathomimetic activity in canine isolated perfused mesenteric vessels. A similar observation has also been made in the rat aorta precontracted with KCl (Doggerell, 1990). In both instances, the vasorelaxant effect of pindolol was not significantly inhibited by propranolol, thus suggesting the presence of an atypical β-adrenoceptor subtype different from the conventional β_1- and β_2-adrenoceptors. In a recent study, it was shown that propranolol antagonized the vasorelaxant effect of isoprenaline in the rat aorta (Gray & Marshall, 1992). However, the K_B value (3×10^{-8} M) was much less than values of $1-3 \times 10^{-9}$ M expected from an action on conventional β_1- and β_2-adrenoceptors. This observation would probably support the presence of atypical β-adrenoceptors in the rat aorta. The present studies were designed to determine the possible existence of β_3-adrenoceptors in vascular smooth muscles of the rat.

Methods

Sprague-Dawley rats of either sex weighing 150–200 g were used in this study. Each rat was anaesthetized with chloroform and bled through a cut in the abdominal aorta. The common carotid arteries were isolated and carefully cleaned of any connective tissue. Thereafter ring segments (3–4 mm) were set up for isometric tension recording, in a 30.0 ml organ bath containing Krebs solution of the following composition (mM): NaCl 119, $NaHCO_3$ 25, KCl 4.7, $MgSO_4$ 1.5, $CaCl_2$, 2.5, KH_2PO_4 1.2 and glucose 11.0. The solution was bubbled continuously with a 95% O_2 and 5% CO_2 gas mixture. The pH was approximately 7.4. To obtain relaxant responses, the arterial segments were contracted with noradrenaline (10^{-7} M). This concentration of noradrenaline usually produced about 85–90% of the maximum contraction in this tissue. Ascending concentrations of the agonists were added cumulatively to generate a concentration-response curve. Unless otherwise stated, the endothelium was maintained intact as shown by the ability of carbachol (10^{-6} M) to relax noradrenaline precontracted ring segments. When antagonists were used, each antagonist concentration was added to the bath and allowed to equilibrate with the tissue for 30 min before the agonist concentration-response curve was repeated. In all cases, only one agonist/antagonist pair was tested on any one preparation and only one antagonist concentration was tested on each preparation. Antagonist potency was expressed as the pA_2 value obtained according to Arunlakshana & Schild (1959). Antagonism was assumed to be competitive when the slope of the Schild regression line was not significantly different from 1. In preliminary experiments, the potency of isoprenaline was not affected by including cocaine (3×10^{-6} M) and deoxycorticosterone acetate (10^{-5} M) in the Krebs solution to block uptake$_1$ and uptake$_2$ respectively. These blockers were therefore not routinely used in this study.

Drug solutions

The following drugs were used in this study; carbachol hydrochloride, (−)-noradrenaline (arterenol) hydrochloride, (±)-propranolol hydrochloride (all from Sigma), (±)-CGP 12177 (4-[3-[1,1-dimethyl(ethyl)amino]-2-hydroxypropoxy]-1, 3-dihydro-2H-benzimidazol-2-one hydrochloride), salbutamol, (±)-isoprenaline (isoproterenol) hydrochloride (RBI) and BRL 37344, (**R*R***)-(±)-[4-[2-[2-(3-chlorophenyl)-2-

[1] Author for correspondence.

hydroxyethylamino]propyl]phenoxyacetic acid (courtesy of Smithkline Beecham, Epsom, Surrey). All the compounds were dissolved in distilled water or dilute hydrochloric acid.

Statistical analysis

Results are presented as mean ± s.e. of n number of experiments. Mean values were compared by Student's t test and differences between mean values were accepted as significant when $P<0.05$.

Results

Isoprenaline (10^{-8} M–10^{-5} M), produced concentration-dependent relaxation of noradrenaline (10^{-7} M) precontracted ring segments of the rat common carotid artery (Figure 1). The pD_2 value was 7.1 ± 0.1 ($n = 8$). In all cases, isoprenaline produced 100% relaxation (of the induced tone) and in some cases, even a little relaxation below the baseline. The relaxation was rapid in onset and the peak response was attained within 90 s. Propranolol (10^{-8} M–3×10^{-7} M) shifted the isoprenaline concentration-response curve to the right without reducing the maximum response (Figure 2). The Schild plot gave a straight line with a slope of 0.74 ± 0.07. As this value was significantly ($P<0.05$) less than 1, the X-intercept (8.85) was therefore strictly not a pA_2 value. Isoprenaline (10^{-8} M–10^{-5} M) relaxed the precontracted ring segments of the carotid artery with or without the endothelium.

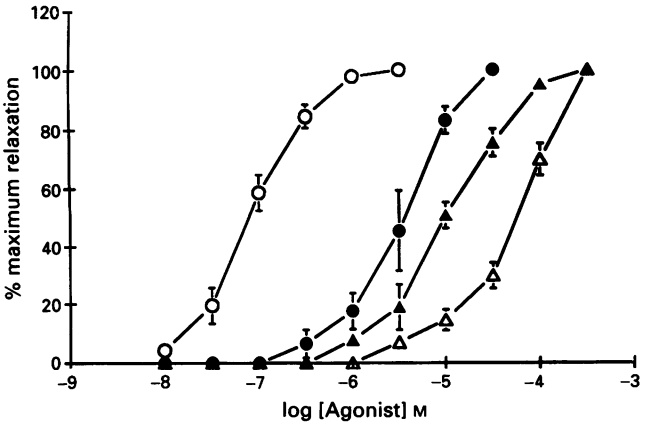

Figure 1 Inhibitory effect of isoprenaline (○), CGP 12177 (●), salbutamol (▲) and BRL 37344 (△) on ring segments of rat common carotid artery precontracted with noradrenaline (10^{-7} M). Each point represents the mean ± s.e. of 4–8 experiments.

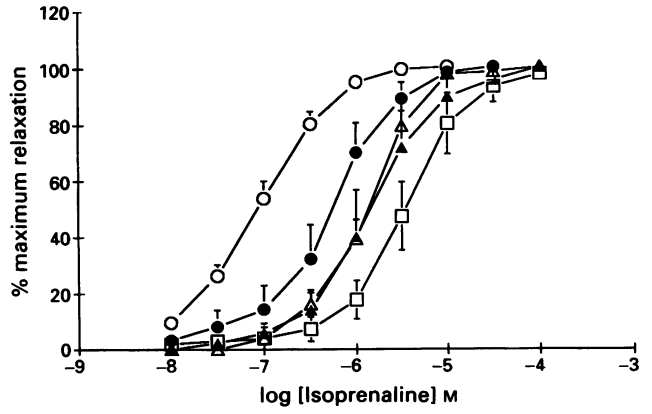

Figure 2 Concentration-response curves for isoprenaline in the absence (○) and in the presence of propranolol 10^{-8} M (●), 3×10^{-8} M (△), 10^{-7} M (▲) and 3×10^{-7} M (□). Each point represents the mean ± s.e. of 4 experiments.

Relaxant effects of selective β-adrenoceptor agonists on the precontracted carotid artery

CGP 12177 (10^{-6} M–3×10^{-5} M), BRL 37344 (10^{-5} M–3×10^{-4} M) and salbutamol (10^{-6} M–10^{-4} M) also concentration-dependently (Figure 1) relaxed ring segments of the carotid artery contracted with noradrenaline (10^{-7} M). The relaxant effects of CGP 12177 and salbutamol were rapid in onset and a peak response was usually attained within 120 s. On the other hand, the relaxant effect of BRL 37344 was slow in onset and the peak response was achieved in about 5–8 min. Removal of the endothelium did not modify the relaxant effect of CGP 12177, BRL 37344 and salbutamol. pD_2 values were 5.4 ± 0.1 ($n = 8$), 4.3 ± 0.1 ($n = 5$) and 5.1 ± 0.1 ($n = 4$) for CGP 12177, BRL 37344 and salbutamol respectively. CGP 12177, BRL 37344 and salbutamol were full agonists in this preparation producing the same maximum response as isoprenaline. Propranolol (10^{-7} M) did not significantly ($P>0.05$) affect the relaxant responses to CGP 12177, BRL 37344 and salbutamol. pD_2 values for these agonists in the absence and also in the presence of propranolol (10^{-7} M) are shown in Table 1. This concentration of propranolol (i.e. 10^{-7} M) produced a concentration-ratio of approximately 30 against isoprenaline.

Effect of pretreatment with BRL 37344 on the relaxant effect of isoprenaline, CGP 12177 and salbutamol

Since it has been consistently observed in non-vascular smooth muscles that pretreatment of the tissues with BRL 37344 induces cross-desensitization to other agonists acting on atypical β-adrenoceptors (e.g. McLaughlin & MacDonald, 1990), an attempt was made to determine whether such a phenomenon can occur in a vascular smooth muscle preparation. This was done by establishing concentration-response curves to isoprenaline, CGP 12177 and salbutamol (in the presence of propranolol, 10^{-7} M, to block β_1- and β_2-adrenoceptor subtypes) before and after BRL 37344 (10^{-4} M). The results show that in artery segments treated with BRL 37344 (10^{-4} M) for 30 min, there was no reduction in the potency of isoprenaline (Figure 3). There was also no change in the maximum response. The relaxant effects of CGP 12177 and salbutamol were also not modified by pretreating the tissues with BRL 37344. pD_2 values for the agonists before and after pretreatment with BRL 37344 (10^{-4} M) are shown in Table 2. As shown in Figure 3, a pre-exposure of the arterial segments to BRL 37344 did not affect the response to a subsequent addition of the same concentration of BRL 37344.

Discussion

β-Adrenoceptors in vascular smooth muscles were initially classified as β_2-adrenoceptors (Lands *et al.*, 1967b). Later studies using more selective agonists and antagonists have shown that vasodilatation could result from activation of β_1- or β_2-adrenoceptor subtypes. For example, while β_2-adrenoceptors predominate in the guinea-pig pulmonary artery (O'Donnell & Wanstall, 1985) and human saphenous vein (Ikezono *et al.*, 1987), β_1-adrenoceptors appear to predominate in the rabbit facial vein (McPherson & Bevan,

Table 1 Effect of propranolol on the relaxant effect of CGP 12177, BRL 37344 and salbutamol in ring segments of the rat common carotid artery precontracted with noradrenaline (10^{-7} M)

	pD_2 values	
Agonist	*Control*	*Propranolol (10^{-7} M)*
CGP 12177	5.4 ± 0.1 ($n = 8$)	5.4 ± 0.1 ($n = 8$)
BRL 37344	4.3 ± 0.1 ($n = 5$)	4.5 ± 0.07 ($n = 6$)
Salbutamol	5.5 ± 0.2 ($n = 4$)	5.4 ± 0.1 ($n = 4$)

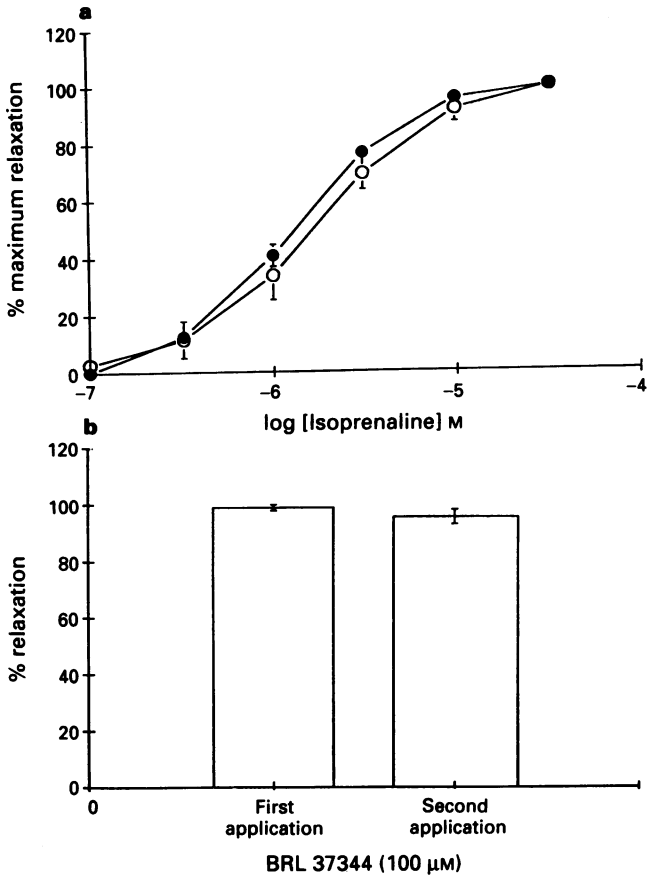

Figure 3 Concentration-response curves for isoprenaline before (○) and after (●) pretreatment with BRL 37344 (10^{-4} M) for 30 min (a) and the effect of pretreatment with BRL 37344 (10^{-4} M) on the relaxant response to a subsequent addition of BRL 37344 (b) on noradrenaline-precontracted rat common carotid artery segments.

Table 2 Effect of pretreatment with BRL 37344 (10^{-4} M, for 30 min) on the relaxant potency of Isoprenaline, CGP 12177 and salbutamol in ring segments of the common carotid artery precontracted with noradrenaline (10^{-7} M)

	pD_2 values	
Agonist	*Before BRL 37344*	*After BRL 37344*
Isoprenaline	5.8 ± 0.1 ($n = 4$)	5.9 ± 0.1 ($n = 4$)
CGP 12177	5.6 ± 0.1 ($n = 4$)	5.6 ± 0.1 ($n = 4$)
Salbutamol	5.4 ± 0.2 ($n = 4$)	5.4 ± 0.2 ($n = 4$)

1987), coronary and cerebral arteries (Edvinsson & Owman, 1974; Edvinsson *et al.*, 1976). In most other vessels, the isoprenaline-induced relaxation is mediated mainly via β_2-adrenoceptor activation with a little contribution from β_1-adrenoceptors (Taira *et al.*, 1977; Cohen & Wiley, 1978; O'Donnell & Wanstall, 1985). Isoprenaline and propranolol are non-selective β_1- and β_2-adrenoceptor agonist and antagonist respectively. It would therefore be expected that propranolol would antagonize isoprenaline competitively. However, results obtained in the present study show that even though propranolol produced a surmountable antagonism of isoprenaline-induced relaxation of precontracted ring segments of the rat common carotid artery, the antagonism did not satisfy the criteria for competitive antagonism. The Schild-plot was a straight line with a slope of 0.74 which was significantly different from 1. These results are therefore consistent with isoprenaline interacting with two sites having high and low affinities for propranolol probably corresponding to β_1- and β_2-adrenoceptor subtypes (high affinity) and atypical (β_3?) β-adrenoceptors (low affinity). It seems unlikely that β_2-adrenoceptors are involved in the relaxant response to isoprenaline in the carotid artery based on the very low potency of salbutamol in this preparation. Salbutamol is a potent and selective β_2-adrenoceptor agonist. pD_2 values reported in the literature vary from 7.0 in the guinea-pig lung strip (Schreurs *et al.*, 1980) to 8.3 in uterine smooth muscle (Granger *et al.*, 1985). The low pD_2 value (approximately 5.0) obtained in the present study is therefore not consistent with activation of β_2-adrenoceptors. This is supported by the fact that the potency ratio isoprenaline:salbutamol is greater than 100, a value that is about 10 fold greater than the usual potency ratio of about 10 observed in tissues containing conventional β_2-adrenoceptors (Arch *et al.*, 1984; Piercy, 1988). The high isoprenaline:salbutamol potency ratio observed in this study is similar to that obtained in brown adipose tissue of the rat (Arch *et al.*, 1984). Confirmation of salbutamol interacting with an atypical rather than conventional β_2-adrenoceptors was provided by the observation that propranolol (10^{-7} M) did not significantly affect salbutamol-induced relaxation of the carotid artery. This concentration of propranolol is at least 10 times its pA_2 value at conventional β_2-adrenoceptor sites. A similar conclusion was reached concerning the rabbit jejunum where propranolol antagonized salbutamol with a pA_2 value of 6.6 (Norman & Leathard, 1990).

CGP 12177 is a potent non-selective β_1- and β_2-adrenoceptor antagonist (Staehelin *et al.*, 1983; Bahouth & Malbon, 1988). It is also an agonist at atypical β-adrenoceptors, stimulating thermogenesis in brown adipose tissues (Mohell & Dickers, 1989) and lipolysis in white adipose tissues (Langin *et al.*, 1991) of the rat. In the present study, CGP 12177 behaved as a full agonist producing the same maximum relaxation of the precontracted carotid artery and was equipotent with isoprenaline (in the presence of propranolol, 10^{-7} M, to block β_1- and β_2-adrenoceptors). The relaxant effect of CGP 12177 was not antagonized by propranolol (10^{-7} M) thus confirming interaction of CGP 12177 with atypical β-adrenoceptors in the carotid artery. Another selective agonist used to demonstrate the presence of atypical β-adrenoceptors in the carotid artery is BRL 37344. This compound is a potent stimulant of lipolysis in both brown and white adipose tissues of the rat (Arch *et al.*, 1984; Wilson *et al.*, 1984). The lipolytic effect of BRL 37344 was not antagonized by propranolol at the usual β_1- and β_2-adrenoceptor blocking doses. BRL 37344 also potently relaxed smooth muscles of the gastrointestinal tract including the rat proximal colon (Bianchetti & Manara, 1990), distal colon (McLaughlin & McDonald, 1990; Kirkham & Kelly, 1992), gastric fundus (McLaughlin & Macdonald, 1991) and oesophagus (de Boer *et al.*, 1993), guinea-pig colon (Coleman *et al.*, 1987) and ileum (Bond & Clarke, 1988). These responses were resistant to antagonism by propranolol. Results obtained in the present study showed that BRL 37344 relaxed the precontracted ring segments of the carotid artery. The relaxation was slow in onset and took a longer time (compared with isoprenaline, CGP 12177 and salbutamol) to attain a peak response. This is similar to the slowly developing relaxation induced by BRL 37344 in smooth muscles of the gastrointestinal tract. However, the potency was very low (5.0) compared to values previously reported for other tissues such as the rat distal colon (McLaughlin & MacDonald, 1990; Kirkham & Kelly, 1992) and oesophagus (de Boer *et al.*, 1993). It is however close to the value obtained in the rat fundus (McLaughlin & MacDonald, 1991). The reason for this rather poor potency is not known.

Cross-desensitization to all agonists is a common occurrence in tissues pretreated with BRL 37344. In the rat distal colon for example, pretreatment of the tissue with BRL 37344 resulted in a rightward shift of the dose-response curve to isoprenaline and BRL 37344 (McLaughlin & MacDonald, 1990). This phenomenon has been interpreted as indicating a common site of action for the agonists. In the present study,

pretreatment of the tissues with BRL 37344 did not reduce either the potency of or the maximum response to isoprenaline, CGP 12177 and salbutamol. This could suggest that the atypical β-adrenoceptors in the carotid artery may differ from that in the gut tissues or that the coupling mechanism is not irreversibly affected by BRL 37344.

It was therefore concluded that there are atypical β-adrenoceptors mediating relaxation in the rat common carotid artery and that CGP 12177 might be a useful compound for identifying such receptors in vascular smooth muscles. The physiological significance of these atypical β-adrenoceptors in the vascular smooth muscle cannot be determined from this set of results. However, the receptor might be involved in the regulation of blood flow to some organs. For example, β_3-adrenoceptor mediated vasodilatation is mainly seen in vessels of the skin and fat in conscious dogs (Shen *et al.*, 1992).

The technical assistance of Mrs Elizabeth A. Kadavil is gratefully acknowledged. The author is also grateful to SmithKline Beecham pharmaceuticals for financial assistance.

References

ARCH, J.R.S. (1989). the brown adipocyte β-adrenoceptor. *Proc. Nutr. Soc.*, **48**, 215–233.

ARCH, J.R.S., AISWORTH, A.T., CAWTHORNE, M.A., PIERCY, V., SENNITT, M.V., THODY, V.E., WILSON, C. & WILSON, S. (1984). Atypical β-adrenoceptors on brown adipocytes as target for anti-obesity drugs. *Nature*, **309**, 363–365.

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

BAHOUTH, S.W. & MALBON, C.C. (1988). Subclassification of beta adrenergic receptors of rat fat cells: a re-evaluation. *Mol. Pharmacol.*, **34**, 318–326.

BIANCHETTI, A. & MANARA, L. (1990). In vitro inhibition of intestinal motility by phenylethanolaminotetralines: evidence for atypical β-adrenoceptors in the rat colon. *Br. J. Pharmacol.*, **100**, 831–839.

DE BOER, R.E.P., BROUWER, F. & ZAAGSMA, J. (1993). The β-adrenoceptors mediating relaxation of rat oesophageal muscularis mucosae are predominantly of the β_3-, but also of the β_2-subtype. *Br. J. Pharmacol.*, **110**, 442–443.

BOND, R.A. & CLARKE, E.C. (1988). Agonist and antagonist characterisation of a putative adrenoceptor with distinct pharmacological properties from the α and β subtypes. *Br. J. Pharmacol.*, **95**, 723–734.

CLARK, B.J. & BERTHOLET, A. (1983). Effects of pindolol on vascular smooth muscle. *Gen. Pharmacol.*, **14**, 117–119.

COHEN, M.L. & WILEY, K.S. (1978). Rat jugular vein relaxes to norepinephrine, phenylephrine and histamine. *J. Pharmacol. Exp. Ther.*, **205**, 400–409.

COLEMAN, R.A., DENYER, L.H. & SHELDRICK, K.E. (1987). β-Adrenoceptors in guinea-pig gastric fundus – are they the same as the atypical β-adrenoceptors in rat adipocytes? *Br. J. Pharmacol.*, **90**, 40P.

DOGGRELL, S.A. (1990). Relaxant and β_2-adrenoceptor blocking activities of (±)-, (+)- and (−)-pindolol on the rat isolated aorta. *J. Pharm. Pharmacol.*, **42**, 444–446.

EDVINSSON, L. & OWMAN, C. (1974). Pharmacological characterization of adrenergic alpha and beta receptors mediating the vasomotor responses of cerebral arteries in vitro. *Circ. Res.*, **35**, 835–849.

EDVINSSON, L., OWMAN, C. & SJOBERG, N.O. (1976). Autonomic nerves, mast cells and amine receptors in human brain vessels: a histochemical and pharmacological study. *Brain Res.*, **115**, 377–393.

GRANGER, S.E., HOLLINGWORTH, M. & WESTON, A.H. (1985). A comparison of several calcium antagonists on uterine, vascular and cardiac muscles from the rat. *Br. J. Pharmacol.*, **85**, 255–262.

GRAY, D.W. & MARSHALL, I. (1992). Novel signal transduction pathway mediating endothelium-dependent relaxation in rat thoracic aorta. *Br. J. Pharmacol.*, **107**, 684–690.

IKEZONO, K., ZERKOWSKI, H.-R., BECKERINGH, J.J., MICHEL, M.C. & BRODDE, O.-E. (1987). Beta-2 adrenoceptor-mediated relaxation of the isolated human saphenous vein. *J. Pharmacol. Exp. Ther.*, **241**, 294–299.

KIRKHAM, D.M. & KELLY, J. (1992). Direct comparison of the rat atypical β-adrenoceptors mediating white adipocyte lipolysis and colonic relaxation. *Br. J. Pharmacol.*, **105**, 231P.

LANDS, A.M., ARNOLD, A., MCAULIFF, J.P., LUDUENA, F.P. & BROWN, T.G. (1967a). Differentiation of receptor systems activated by sympathomimetic amines. *Nature*, **214**, 597–598.

LANDS, A.M., LUDUENA, F.P. & BUZZO, H.J. (1967b). Differentiation of receptors responsive to isoproterenol. *Life Sci.*, **6**, 2241–2249.

LANGIN, D., PORTILLO, M.P., SAULINER-BLACHE, J.S. & LAFONTAN, M. (1991). Coexistence of three beta-adrenergic receptor subtypes in white fat cells of various mammalian species. *Eur. J. Pharmacol.*, **199**, 291–301.

MCLAUGHLIN, D.P. & MACDONALD, A. (1990). Evidence for the existence of atypical β-adrenoceptors (β_3-adrenoceptors) mediating relaxation in the rat distal colon. *Br. J. Pharmacol.*, **101**, 569–574.

MCLAUGHLIN, D.P. & MACDONALD, A. (1991). Characterization of catecholamine-mediated relaxations in rat isolated fundus: evidence for an atypical β-adrenoceptor. *Br. J. Pharmacol.*, **103**, 1351–1356.

MCPHERSON, G.A. & BEVAN, J.A. (1987). Specialization in beta-1 and beta-2 adrenoceptor distribution in veins of the rabbit face: relationship to myogenic tone and sympathetic nerve innervation. *J. Pharmacol. Exp. Ther.*, **240**, 99–105.

MOHELL, N. & DICKER, A. (1989). The beta adrenergic radioligand [3]CGP 12177, generally classified as an antagonist, is a thermogenic agonist in brown adipose tissue. *Biochem. J.*, **261**, 401–405.

NORMAN, B.J. & LEATHARD, H.L. (1990). Evidence that an atypical β-adrenoceptor mediates the inhibition of spontaneous rhythmical contractions of rabbit isolated jejunum induced by ritodrine and salbutamol. *Br. J. Pharmacol.*, **101**, 27–30.

O'DONNELL, S.R. & WANSTALL, J.C. (1985). Responses to the β_2-selective agonist procaterol of vascular and atrial preparations with different functional β-adrenoceptor populations. *Br. J. Pharmacol.*, **84**, 227–235.

PIERCY, V. (1988). Method for assessing the activity of drugs at β_1- and β_2-adrenoceptors in the same animal. *J. Pharmacol. Method*, **20**, 125–133.

SCHREURS, A.J.M., TERPSTRA, G.K., RAAIJMAKERS, J.A.M. & NIJKAMP, F.P. (1980). Effects of vaccination with Haemophilus influenza on adrenoceptor function of tracheal and parenchymal strips. *J. Pharmacol. Exp. Ther.*, **215**, 691–696.

SHEN, Y.-T., MANDERS, W.T., ZHANG, H. & VATNER, S.F. (1992). β3-Adrenergic receptor peripheral vascular regulation in conscious dogs. *Circulation*, **86**, Suppl. 1, I-297.

STAEHELIN, M., SIMONS, P., JAEGGI, K. & WIGGER, N. (1983). CGP 12177 A hydropholic beta-adrenergic receptor radioligand reveals high affinity binding of agonists to intact cells. *J. Biol. Chem.*, **258**, 3496–3502.

TAIRA, N., YABUUCHI, Y. & YAMASHITA, S. (1977). Profile of beta-adrenoceptors in femoral, superior mesenteric and renal vascular beds of dogs. *Br. J. Pharmacol.*, **59**, 577–583.

WILSON, C., WILSON, S., PIERCY, V., SENNITT, M.V. & ARCH, J.R.S. (1984). The rat lipolytic beta-adrenoceptor: studies using novel beta-adrenoceptor agonists. *Eur. J. Pharmacol.*, **100**, 309–319.

(Received February 1, 1994
Revised May 20, 1994
Accepted June 16, 1994)