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Characterization of catecholamine-mediated relaxations in rat isolated gastric fundus: evidence for an atypical β-adrenoceptor

[1]Daniel P. McLaughlin & [2]Allan MacDonald

Department of Biological Sciences, Glasgow College, Cowcaddens Road, Glasgow G4 0BA

1 Experiments were carried out in order to characterize the receptors mediating relaxant responses to catecholamines in the rat gastric fundus. The effects of noradrenaline, isoprenaline and the 'atypical' or β_3-adrenoceptor agonist, BRL 37344, on methacholine-induced tone were measured. Prazosin, propranolol and cyanopindolol were used as antagonists.

2 Relaxant responses to noradrenaline, in the presence of propranolol (1 μM) were antagonized in a concentration-dependent manner by prazosin (0.01 to 1 μM), although this antagonism was weak and non-competitive in nature. Relaxant responses to isoprenaline, in the presence of prazosin (0.1 μM), were antagonized only by the highest concentration of propranolol (1 μM) giving a pK_B of 6.3. BRL 37344 also relaxed the rat gastric fundus in the presence of prazosin (0.1 μM), and the responses to BRL 37344 were unaffected by propranolol (1 μM).

3 Tachyphylaxis to BRL 37344 was observed, a second concentration-response curve being significantly shifted to the right. Exposure of tissues to BRL 37344 (1 μM) between concentration-response curves also caused an 11 fold rightward shift in the response to isoprenaline.

4 In the presence of prazosin (0.1 μM) and propranolol (1 μM), the rank order of potency of the agonists was: (−)-isoprenaline (1.0) > (−)-noradrenaline (0.39) > BRL 37344 (0.10).

5 Responses to BRL 37344 in the presence of prazosin (0.1 μM) and propranolol (1 μM) were antagonized by (±)-cyanopindolol (1 μM), with a pK_B of 6.56. Responses to isoprenaline, under the same conditions, were antagonized in a competitive manner by (±)-cyanopindolol (0.1–1 μM), with the slope of a Schild plot close to unity and a pA_2 value of 7.44.

6 The resistance to blockade by prazosin and propranolol and the antagonism by cyanopindolol of the responses mediated by isoprenaline and BRL 37344 suggest that atypical β-adrenoceptors similar to 'atypical' β-adrenoceptors in rat adipocytes and other tissues are present in the rat gastric fundus.

Keywords: Atypical β-adrenoceptors; catecholamines; smooth muscle; gastric fundus

Introduction

It is well known that catecholamines generally produce relaxation of non-sphincteric gastrointestinal smooth muscle and that this effect is mediated by postjunctional α- and β-adrenoceptors (Burnstock & Wong, 1981). However, relaxant responses to catecholamines which are resistant to blockade by α- and β-adrenoceptor antagonists have also been reported in a number of gastrointestinal smooth muscle preparations including rabbit stomach (Bristow *et al.*, 1970), rabbit colon (Gillespie & Khoyi, 1977), rat oesophageal smooth muscle (Buckner & Christopherson, 1974), guinea-pig ileum (Wikberg, 1977; Bond *et al.*, 1986; Bond & Clarke, 1987), dog colon (Grivegnee *et al.*, 1984), rat gastric fundus (Dettmar *et al.*, 1986; McLaughlin & MacDonald, 1990a; Kelly & MacDonald, 1990), rat proximal colon (Croci *et al.*, 1988; Manara *et al.*, 1989; Bianchetti & Manara, 1990), rabbit jejunum (Norman & Leathard, 1990), rat jejunum (Van der Vliet *et al.*, 1990) and rat distal colon (McLaughlin & MacDonald, 1989; 1990b). In the guinea-pig ileum, the order and relative potency of catecholamines is consistent with β-adrenoceptors although responses were resistant to propranolol (Bond & Clarke, 1988). In addition, the receptor mediating propranolol-resistant responses is characterized by the relatively high potency of a novel class of β-adrenoceptor agonists (Arch *et al.*, 1984), e.g. BRL 37344 in guinea-pig ileum (Bond & Clarke, 1988) and rat distal colon (McLaughlin & MacDonald, 1990b) and BRL 35135 in guinea-pig gastric fundus (Coleman *et al.*, 1987).

Atypical β-adrenoceptors also exist in non-gastrointestinal tissue, for example in skeletal muscle (Challis *et al.*, 1988) and in heart (Kaumann, 1989), and resistance of catecholamine-mediated responses to classical β-adrenoceptor antagonists and the high potency of BRL 37344 are also features of the atypical β-adrenoceptors mediating lipolysis in rat adipocytes (Stanton, 1972; Harms *et al.*, 1977). It has been suggested that these adipocyte β-adrenoceptors may represent a separate subclass of β-adrenoceptor, which has been termed the 'β_3-adrenoceptor' (Tan & Curtis-Prior, 1983; Arch, 1989). Recently a human gene has been isolated that encodes a product with similar properties (low affinity for classical β-adrenoceptor antagonists and high potency of BRL 37344) and referred to as the 'β_3-adrenergic receptor' (Emorine *et al.*, 1989).

The aim of the present study was to investigate further the prazosin- and propranolol-resistant responses to catecholamines previously reported in rat gastric fundus (Dettmar *et al.*, 1986; Kelly & MacDonald, 1990) by comparing the potencies of isoprenaline, noradrenaline and BRL 37344. In addition the effects of the β-adrenoceptor antagonist cyanopindolol, which has been reported to be a competitive antagonist at atypical β-adrenoceptors in guinea-pig ileum (Blue *et al.*, 1989), were investigated.

A preliminary account of this work has been presented to the British Pharmacological Society (McLaughlin & MacDonald, 1990a).

Methods

Tissue preparation

Male Wistar rats (250–350 g), fed *ad libitum*, were killed by a blow to the head and cervical dislocation. Longitudinal strips of gastric fundic smooth muscle (15 mm long, 3 mm wide) were dissected by the method of Vane (1957) and were suspended in

[1] Present address: Department of Neuroscience, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London.
[2] Author for correspondence.

organ baths in 30 ml Krebs physiological saline solution (PSS) under an initial isometric tension of 1 g. The Krebs PSS was maintained at 37°C and was bubbled continuously with a mixture of 95% O_2 and 5% CO_2. The composition of the Krebs PSS was as follows (mM): NaCl 118, $CaCl_2$ 2.5, KCl 4.7, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2 and glucose 11.1. The Krebs solution contained cocaine (3 μM) to block neuronal uptake of noradrenaline and ascorbic acid (30 μM) and EDTA (30 μM) to offset oxidation of the catecholamines. Tissues were allowed to equilibrate for a period of at least 30 min before experimental procedures were begun.

Concentration-response curves to noradrenaline were constructed in the presence of propranolol (1 μM) to allow investigation of the contribution of α-adrenoceptors to responses. Concentration-response curves to isoprenaline were carried out in the presence of prazosin (0.1 μM) to remove any contribution from α_1-adrenoceptors.

Concentration-response curves (CRCs)

The relaxant action of the agonists was determined by measuring the percentage relaxation of methacholine (MCh)-induced tone produced by an agonist. Initially, a MCh CRC was constructed for each tissue, to determine a concentration producing approximately 50% of maximal tone. In most cases, this was between 0.3 and 1 μM. CRCs to agonists were constructed by cumulative addition (0.5 log unit increments) to MCh-contracted strips until a stable response was observed. In antagonist studies of isoprenaline and noradrenaline, four consecutive CRCs were constructed at 1 h intervals; one control CRC followed by three CRCs with increasing concentration of antagonist. Antagonists were added at the end of each CRC, allowing 45 min equilibration time. Some tissues received no antagonist and served as controls to assess time-dependent changes. In antagonist studies with BRL 37344 only one CRC to BRL 37344 could be carried out in any one tissue due to desensitization. In these experiments tissues were exposed to BRL 37344 in the presence and absence of antagonist.

Schild plots

Agonist concentration-ratios (CRs) were determined from EC_{50} values with or without antagonist and were corrected for time-dependent effects by dividing by the apparent shift in tissues in which no antagonist was present.

The plot of log (agonist concentration ratio − 1.0) versus log [antagonist] (Arunlakshana & Schild, 1959) was analysed by linear regression. Antagonism was considered to be simple competitive in nature if the slope of the regression line was not significantly different from unity. In such cases a mean pA_2 value was obtained from individual estimates from the equation:

$$pA_2 = \log(\text{agonist CR} - 1) - \log[\text{antagonist}]$$

after verifying that there was no significant regression of pA_2 on antagonist concentration (MacKay, 1978).

In some experiments only one concentration of antagonist was tested and in these cases the value obtained from the above equation is a pK_B rather than a pA_2 value.

Drugs used

The following drugs were dissolved in distilled water (with the exception of (±)-cyanopindolol which was dissolved in 0.1 M tartaric acid): acetyl-β-methyl choline (methacholine) chloride (Sigma); BRL 37344 (sodium-4-[-2-[2-hydroxy-2-(-3-chlorophenyl) ethylamino] propyl] phenoxyacetate) (Beecham Research Laboratories); cocaine hydrochloride (Thornton and Ross Ltd.); (±)-cyanopindolol (Sandoz); (−)-isoprenaline (+)-bitartrate (Sigma); (−)-noradrenaline (+)-bitartrate (arterenol) (Sigma); prazosin hydrochloride (synthesized by Reckitt and Colman plc); (±)-propranolol hydrochloride (Sigma).

Statistical analysis

Results are expressed as mean ± s.e.mean with the number of observations, n, in parentheses with the exception of regression line slopes which are expressed as slope ± 95% confidence limits. Statistical significance between two data sets was tested by either Student's t test or a paired t test. A probability level of $P < 0.05$ was considered to be statistically significant.

Results

Responses to noradrenaline

In the presence of propranolol (1 μM), noradrenaline relaxed MCh-induced tone in the rat gastric fundus, with a pD_2 of 6.7 ± 0.15 ($n = 8$). Responses to noradrenaline consisted of two phases (Figure 1a). An initial, 'sharp' phase was followed by a secondary partial recovery of the tone. The initial 'sharp' phase of relaxation in response to noradrenaline was prevented by prazosin (0.01 to 0.1 μM, Figures 1b and c). The relaxant responses to noradrenaline were reproducible with time, and no statistically significant time-dependent decrease in the sensitivity of tissues to noradrenaline was observed over the 3 h time period (Figure 2a).

CRCs to noradrenaline were antagonized in a concentration-dependent manner by prazosin (0.01 to 1 μM, Figure 2b). A Schild plot (Figure 2c) revealed that this antagonism did not appear to be simple competitive in nature, the slope of the plot being significantly less than unity (0.53 ± 0.28, 95% C.L., $n = 23$).

Responses to isoprenaline

In the presence of prazosin (0.1 μM), isoprenaline relaxed MCh-tone, with a pD_2 of 7.7 ± 0.21 ($n = 6$). Responses to iso-

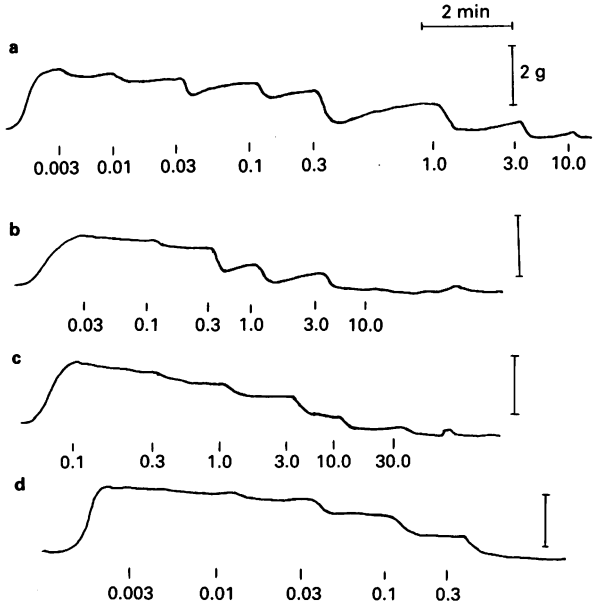

Figure 1 Representative tracings showing relaxations of rat isolated gastric fundus elicited by catecholamines. In each case tone was induced by addition of 1 μM methacholine. Lines indicate addition of catecholamines and values are concentration (μM). (a) Noradrenaline in the presence of propranolol (1 μM); (b) noradrenaline in the presence of propranolol (1 μM) and prazosin (0.01 μM); (c) noradrenaline in the presence of propranolol (1 μM) and prazosin (0.01 μM); (d) isoprenaline in the presence of prazosin (0.1 μM).

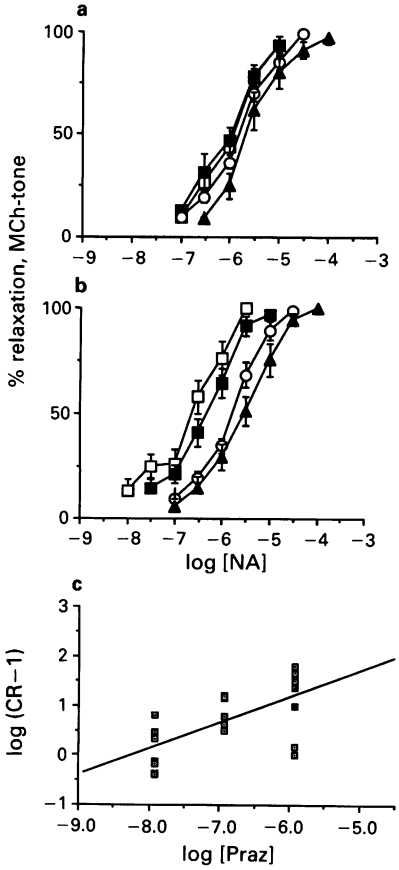

Figure 2 The effect of prazosin on concentration-response curves to noradrenaline in the rat gastric fundus in the presence of propranolol (1 μM). Tone was raised by the addition of methacholine (1 μM) to the organ baths. (a) Time control responses to noradrenaline. First (□, $n = 4$), second (■, $n = 4$), third (○, $n = 4$) and fourth (▲, $n = 4$) concentration-response curves to noradrenaline, constructed at hourly intervals. (b) Effect of prazosin (Praz): (□), controls ($n = 8$); (■), 0.01 μM prazosin ($n = 8$); (○), 0.1 μM prazosin ($n = 8$); (▲), 1 μM prazosin ($n = 8$). (c) The Schild plot of data from (b).

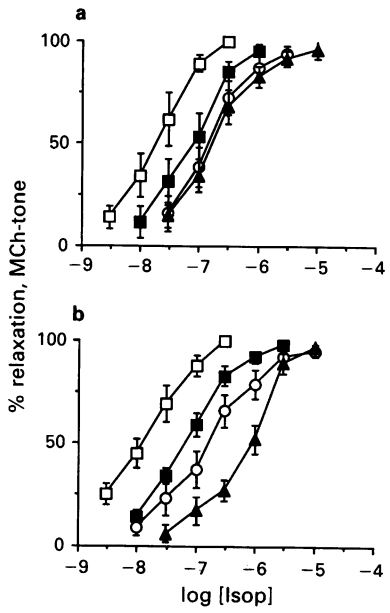

Figure 3 The effect of propranolol on concentration-response curves to isoprenaline (Isop) in the rat gastric fundus in the presence of prazosin (0.1 μM). Tone was raised by the addition of methacholine (1 μM) to the organ baths. (a) Time control responses to isoprenaline. First (□, $n = 4$), second (■, $n = 4$), third (○, $n = 4$) and fouth (▲, $n = 4$) concentration-response curves to isoprenaline, constructed at hourly intervals. (b) Effect of propranolol. (□), controls ($n = 6$); (■), 0.01 μM propranolol ($n = 6$); (○), 0.1 μM propranolol ($n = 6$); (▲), 1 μM propranolol ($n = 6$).

prenaline consisted of a slow relaxation of the tissue with no initial 'sharp' phase of relaxation as seen with noradrenaline (Figure 1d). A time-dependent decrease in the sensitivity of the tissues to isoprenaline (Figure 3a) was taken into consideration when calculating shifts produced by propranolol (see Methods). After correction for time-dependent changes, the shifts produced by propranolol 0.01 and 0.1 μM were insignificant (Figure 3b). Propranolol, 1 μM, did produce a significant shift of the CRC to isoprenaline (Figure 3b, 3.5 fold shift of pD_2 value, $P < 0.05$) giving a pK_B of 6.30 ± 0.14 ($n = 6$).

Responses to BRL 37344

In the presence of prazosin (0.1 μM), BRL 37344 relaxed MCh-induced tone with a pD_2 of 5.22 ± 0.09 ($n = 8$). Propranolol (1 μM) had no effect on the CRC to BRL 37344, the pD_2 of BRL 37344 being 5.30 ± 0.15 ($n = 15$) in the presence of prazosin and propranolol (1 μM).

Tachyphylaxis to BRL 37344 was observed, although this was difficult to quantify, since in second CRCs the highest concentration of BRL 37344 which could be produced in the organ baths (100 μM) often failed to produce even 30% relaxation of the tissue (Figure 4a). In the presence of prazosin (0.1 μM) and propranolol (1 μM), exposure of the tissue to BRL 37344 (1 μM) for a 3 min period between CRCs to isoprenaline produced a significant rightward shift in the CRC to isoprenaline (mean 11 fold shift of pD_2 values, $P < 0.01$, Figure 4b).

Order of potency of agonists in the presence of α- and β-adrenoceptor blockade

In the presence of prazosin (0.1 μM) and propranolol (1 μM), the pD_2 values of isoprenaline, noradrenaline and BRL 37344 were respectively, 6.30 ± 0.17 ($n = 6$), 5.89 ± 0.08 ($n = 8$) and

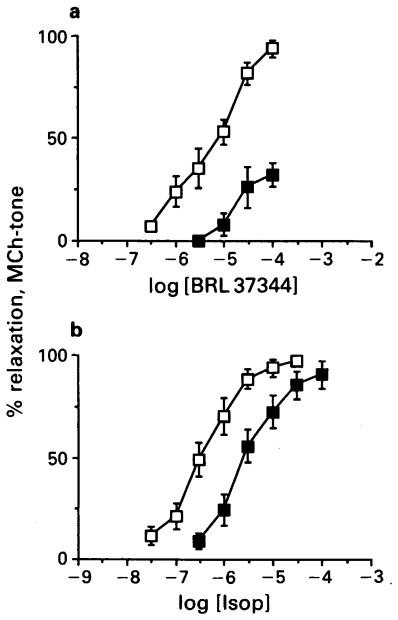

Figure 4 Tachyphylaxis and cross-desensitization to BRL 37344 in the rat gastric fundus in the presence of prazosin (0.1 μM) and propranolol (1 μM). (a) First (□, $n = 5$) and second (■, $n = 5$) concentration-response curves to BRL 37344, constructed at an interval of 1 h. (b) The effect of exposure of portions of gastric fundus to 1 μM BRL 37344 for a 3 min period between concentration-response curves to isoprenaline: mean concentration-response curves to isoprenaline (Isop) before (□, $n = 8$) and after (■, $n = 8$) exposure.

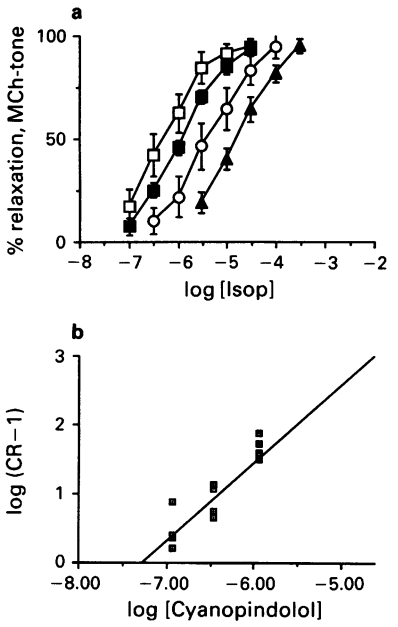

Figure 5 The effect of cyanopindolol (0.1 to 1 μM) on mean concentration-response curves to isoprenaline (Isop) in the rat gastric fundus in the presence of 0.1 μM prazosin and 1 μM propranolol. (a) Mean concentration-response curves to isoprenaline in the absence (□, $n = 4$) and presence of 0.1 μM (■, $n = 4$), 0.3 μM (○, $n = 4$) and 1 μM (▲, $n = 4$) cyanopindolol. (b) Schild plot of the antagonism of isoprenaline action by cyanopindolol.

5.30 ± 0.15 ($n = 15$), giving a rank order of potency of isoprenaline (1.0) > noradrenaline (0.39) > BRL 37344 (0.10).

Effect of cyanopindolol on responses to BRL 37344 and isoprenaline under conditions of α- and β-adrenoceptor blockade

In the presence of prazosin (0.1 μM) and propranolol (1 μM), responses to BRL 37344 were antagonized by cyanopindolol (1 μM) (mean pD_2 values in absence and presence of cyanopindolol in paired tissues: 5.55 ± 0.40 and 4.75 ± 0.25, $n = 5$, $P < 0.05$) giving a pK_B for cyanopindolol against BRL 37344 of 6.56 ± 0.32 ($n = 5$). Also, cyanopindolol (0.1 to 1 μM) antagonized responses to isoprenaline in a concentration-dependent manner (Figure 5a). The antagonism appeared to be simple competitive in nature, since the slope of the Schild plot (Figure 5b) was not significantly different from unity (1.22 ± 0.37, 95% CL, $n = 12$). The pA_2 value for cyanopindolol was 7.44 ± 0.07 ($n = 12$), significantly higher than the pK_B of 6.56 obtained with BRL 37344 as the agonist ($P < 0.001$).

Effect of cyanopindolol on muscle tone

In the experiments described above, cyanopindolol pretreatment had no effect on the degree of tone induced by MCh (tone in cyanopindolol-treated strips as % of control, $n = 4$: 0.1 μM, 123 ± 3; 0.3 μM, 113 ± 6; 1 μM, 97 ± 9).

In separate experiments in which cyanopindolol was added to MCh-contracted strips, higher concentrations than those used for antagonist studies produced some relaxation (% relaxation of MCh-contracted strips by cyanopindolol, $n = 4$: 3 μM, 1 ± 1; 10 μM, 6 ± 4; 30 μM, 21 ± 6; 100 μM, 33 ± 8).

Discussion

In the presence of propranolol to block classical β-adrenoceptors, noradrenaline produced relaxant responses of the rat gastric fundus which were rapid in onset and poorly maintained, a pattern shown previously to occur with α-adrenoceptor-mediated inhibition in rabbit intestine (Bowman & Hall, 1970). The response to noradrenaline was antagonized by prazosin, confirming the presence of postjunctional inhibitory α_1-adrenoceptors reported previously (Verplanken *et al.*, 1984; Dettmar *et al.*, 1986; Kelly & MacDonald, 1990). However, although postjunctional α_2-adrenoceptors are absent from this tissue (MacDonald *et al.*, 1990), the action of noradrenaline could not be attributed wholly to an action at α_1-adrenoceptors since the antagonism by prazosin was not simple competitive, as seen by the low slope of the Schild plot. Verplanken *et al.* (1984) also reported non-competitive antagonism of noradrenaline (but not phenylephrine) by prazosin in the presence of propranolol in rat gastric fundus and suggested that high concentrations of noradrenaline might be overcoming propranolol blockade. This seems unlikely since raising the propranolol concentration from 2 μM to 30 μM failed to affect the prazosin-resistant response (Kelly & MacDonald, 1990). In any case, the previous findings of a prazosin-resistant component of the response to noradrenaline in the presence of propranolol are confirmed (Verplanken *et al.*, 1984; Dettmar *et al.*, 1986; Kelly & MacDonald, 1990).

In the presence of prazosin to block α_1-adrenoceptors, isoprenaline relaxed the fundal strips with a response that was slow in onset and well maintained, characteristic of β-adrenoceptor-mediated inhibition in the gut (Bowman & Hall, 1970). This response was antagonized by propranolol. Lefebvre *et al.* (1984) also reported antagonism of isoprenaline by propranolol and by selective β_1- and β_2-adrenoceptor antagonists. It seems therefore that at least part of the response to isoprenaline in this preparation may be mediated by classical β_1- and β_2-adrenoceptors. However the antagonism produced by propranolol was weak, with a significant shift of the CRC occurring only with the highest concentration (1 μM). The pK_B of 6.3, calculated from the single (1 μM) concentration of propranolol, is low compared with 8.2–8.8 for classical β_1-adrenoceptors mediating atrial stimulation and 8.3–8.6 for β_2-adrenoceptors mediating tracheal relaxation (Wilson *et al.*, 1984) suggesting an action at atypical β-adrenoceptors. A similarly low pK_B was reported for propranolol at atypical β-adrenoceptors mediating lipolysis (6.6, Wilson *et al.*, 1984) or relaxation in rat distal colon (6.57, McLaughlin & MacDonald, 1990b).

BRL 37344, a selective agonist at β_3-adrenoceptors in rat adipocytes (Arch *et al.*, 1984), also produced relaxation which was resistant to antagonism by propranolol, suggesting similarities between the receptor mediating relaxation in the rat gastric fundus and that mediating lipolysis.

BRL 37344 caused tachyphylaxis to itself and to isoprenaline. Tachyphylaxis was previously reported in guinea-pig gastric fundus with BRL 35135, the methyl ester of BRL 37344, (Coleman *et al.*, 1987) and with BRL 37344 in rat distal colon (McLaughlin & MacDonald, 1990b). The tachyphylaxis does not appear to be a non-specific effect since it has been shown previously in rat distal colon that relaxant responses to papaverine were unaffected (McLaughlin & MacDonald, 1990b). The significance of the tachyphylaxis is unclear since it has not been reported to occur in studies of lipolysis.

The relaxant effects of BRL 37344 and isoprenaline in the presence of prazosin and propranolol were antagonized by cyanopindolol. A Schild regression was not carried out for BRL 37344 but the antagonism of isoprenaline by cyanopindolol was consistent with competitive antagonism with the Schild slope close to unity. The mean pA_2 value of 7.44 is similar to the value of 7.63 reported by Blue *et al.* (1989) for cyanopindolol in guinea-pig ileum and to the value of 7.12 in rat distal colon (McLaughlin & MacDonald, 1990b). Thus the receptor mediating responses to isoprenaline in rat gastric fundus appears similar to the atypical β-adrenoceptor in guinea-pig ileum and rat distal colon. The pK_B calculated from the single concentration of cyanopindolol against BRL 37344 (6.56) was significantly lower than the pA_2 value obtained with isoprenaline as agonist (7.44). Lower pA_2 values

for antagonists against BRL compounds have previously been shown (Stock & Sudera, 1988; Jones *et al.*, 1989; Arch, 1989; McLaughlin & MacDonald, 1990b) and attributed to allosteric modification of the binding site by BRL compounds, resulting in lowered antagonist affinity (Arch, 1989).

Cyanopindolol itself produced relaxation of the rat gastric fundus at high concentrations. Some β-adrenoceptor antagonists including pindolol analogues, have previously been shown to act as partial agonists in rat adipocytes (Engel *et al.*, 1981) and at atypical β-adrenoceptors in the heart (Walter *et al.*, 1984; Kaumann, 1989). In the heart the agonism occurred at concentrations higher than required for blockade of conventional β_1- and β_2-adrenoceptors, suggesting an action at a third site, possibly the atypical β-adrenoceptor. However, in the present study the effect of cyanopindolol was only obtained at concentrations considerably higher than those producing blockade of the propranolol-resistant responses. As partial agonists should produce agonist effects at the same concentrations as blockade if the same receptor is involved (Kenakin, 1987), this would suggest that the partial agonist activity of cyanopindolol is at a receptor other than the atypical β-adrenoceptor. On the other hand, pindolol also acted as a partial agonist of adenosine 3':5'-cyclic monophosphate (cyclic AMP) accumulation in Chinese hamster ovary cells containing no β_1- or β_2-adrenoceptors and transfected with the human gene encoding the 'β_3-adrenergic receptor' and this action occurred at concentrations approximately 14 times higher than the dissociation constant at the β_3-site obtained from receptor binding (Emorine *et al.*, 1989). Thus there may be a significant threshold effect involved in the response (over 50% receptor occupancy required before a response is observed) (Kenakin, 1987). It is therefore possible that the agonist effect of high concentrations of cyanopindolol observed in the present study could be due to a similar phenomenon and is at atypical β-adrenoceptors.

The relative potency of BRL 37344 under conditions of α- and β-adrenoceptor blockade, isoprenaline (1.0) > noradrenaline (0.39) > BRL 37344 (0.10), is less than that reported at rat adipocyte β_3-adrenoceptors (5 fold more potent than isoprenaline, Arch *et al.*, 1984) or at atypical β-adrenoceptors in guinea-pig ileum (2.5 fold more potent than isoprenaline, Bond & Clarke, 1988) or rat distal colon (equipotent with isoprenaline, McLaughlin & MacDonald, 1990b). This may be due to tissue factors, such as efficiency of receptor coupling, which can affect agonist potency ratios (Kenakin, 1987). Alternatively the atypical β-adrenoceptor in rat gastric fundus may not be identical with atypical β-adrenoceptors reported elsewhere. There is ample evidence for differences in sites labelled as atypical e.g. alprenolol antagonized responses to isoprenaline at atypical β-adrenoceptors in rat proximal colon with a pA_2 value of 7.6 (Bianchetti & Manara, 1990), was more than an order of magnitude less potent (pA_2 6.5) at atypical β-adrenoceptors in guinea-pig ileum (Blue *et al.*, 1990) and failed to antagonize cyclic AMP accumulation by isoprenaline in Chinese hamster ovary cells transfected with the 'β_3-adrenergic receptor' gene (Emorine *et al.*, 1989). Further examples of discrepancies (Zaagsma & Nahorski, 1990) suggest that the atypical β-adrenoceptor may represent a class of receptor rather than a single receptor subtype. An interesting proposal by Clarke and co-workers (Blue *et al.*, 1990) is that the atypical β-adrenoeptor may represent a population of β_1-type receptor sites located in a different domain of the receptor molecule. These authors propose that such a site would not differ from the β_1-site in recognition of small agonist molecules (noradrenaline, adrenaline, isoprenaline) binding only to the receptor site, but large molecules with additional binding sites such as antagonists and synthetic agonists would have different affinities for the two sites due to differences in the domain of the receptor site. Further pharmacological characterization of atypical β-adrenoceptors in different locations is required.

In conclusion, the resistance of β-adrenoceptor relaxant responses to propranolol and the antagonism of BRL 37344 and isoprenaline by cyanopindolol suggest the presence of atypical β-adrenoceptors in rat gastric fundus. The relatively low potency of BRL 37344 at this site compared with other atypical β-adrenoceptors may be due to differences in coupling efficiency or may indicate receptor heterogeneity.

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