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Evidence that an atypical β-adrenoceptor mediates the inhibition of spontaneous rhythmical contractions of rabbit isolated jejunum induced by ritodrine and salbutamol

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1 The nature of the adrenoceptors mediating the inhibitory action of noradrenaline, ritodrine and salbutamol on the spontaneous activity of longitudinal muscle of the rabbit jejunum *in vitro* was investigated by use of a range of adrenoceptor antagonists.

2 The actions of ritodrine and salbutamol were antagonized competitively by propranolol. The pA_2 values of 6.4 and 6.6 respectively were smaller than those found elsewhere for β_1- and β_2-adrenoceptors.

3 In contrast, the responses to ritodrine and salbutamol were antagonized only by high concentrations ($>2.7\,\mu M$) of phentolamine and were unaffected by yohimbine ($2.6\,\mu M$), mepyramine ($2.5\,\mu M$) or cimetidine ($4.0\,\mu M$).

4 Ritodrine which is less potent than salbutamol in tissues with typical β_2-adrenoceptors was found to be 8 times more potent than salbutamol in the rabbit jejunum.

5 It is suggested that in the rabbit jejunum ritodrine and salbutamol may act at an atypical β-adrenoceptor, at which propranolol is a competitive but not very potent antagonist.

Introduction

Catecholamines inhibit spontaneous, rhythmical contractions of longitudinal muscle of the rabbit small intestine (Finkleman, 1930), and this response is mediated by both α- and β-adrenoceptors (Furchgott, 1960; Bowman & Hall, 1970). These β-adrenoceptors were initially classified as β_1 (Lands *et al.*, 1967) but, when more selective β_1 and β_2 agonists and antagonists became available, evidence was found for the presence of β_2-adrenoceptors also (Wagner *et al.*, 1981). Nevertheless, certain paradoxes remained unresolved; for example Bowman & Hall (1970) found that noradrenaline exhibited a higher degree of β-selectivity than adrenaline in the rabbit small intestine, and Wagner *et al.* (1981) obtained several findings which are hard to reconcile with typical β_1- or β_2-adrenoceptor-mediated activity. In the present study the nature of the adrenoceptors mediating inhibitory effects of sympathomimetic drugs in the rabbit small intestine have been investigated further by use of noradrenaline, two selective β_2-adrenoceptor agonists (salbutamol and ritodrine) and various antagonists.

Methods

New Zealand White rabbits of either sex, weighing 0.8–0.95 kg, were killed by a blow on the neck and subsequent exsanguination. Segments of jejunum, 1 cm long, were suspended in isolated organ baths containing Krebs solution at 37°C, equilibrated with 95% O_2 and 5% CO_2. The aboral end of each segment was fixed near the bottom of the bath and the oral end was connected via a thread to an isotonic transducer, counterbalanced by a 1 g load, enabling longitudinal muscle activity to be recorded. Each segment was allowed to equilibrate for 45 min by which time regular spontaneous activity was seen and consistent inhibitory responses to submaximal concentrations of noradrenaline were obtained.

Concentration-response curves to noradrenaline, ritodrine or salbutamol were performed in the absence and then in the presence of 3 or more concentrations of either propranolol or phentolamine, each equilibrated for 20 min. Control tissues were treated similarly but were not exposed to an antagonist.

The responses to submaximal concentrations of ritodrine ($0.3\,\mu M$) and salbutamol ($3.3\,\mu M$) were also recorded in the presence of yohimbine ($2.6\,\mu M$), mepyramine ($2.5\,\mu M$) and cimetidine ($4.0\,\mu M$), to test whether their inhibitory action might be mediated through α_2-adrenoceptors or H_1- or H_2-receptors.

Drugs and solutions

The following drugs were used: cimetidine (Smith Kline & French), mepyramine (May & Baker), (−)-noradrenaline bitartrate (Sigma), phentolamine mesylate (Ciba), (±)-propranolol hydrochloride (Sigma), ritodrine hydrochloride (Duphar), salbutamol (Sigma) and yohimbine (Sigma).

All agonists were dissolved and diluted in acid saline (NaCl 161 mM, HCl $10\,\mu M$ and ascorbic acid 0.6 mM). All antagonists were dissolved in saline (NaCl 161 mM) and subsequently diluted in Krebs solution.

The Krebs solution had the following composition (mM): NaCl 118.4, KCl 4.7, $CaCl_2$ 2.6, KH_2PO_4 1.2, $MgSO_4 7H_2O$ 1.2, $NaHCO_3$ 23.8 and glucose 11.1.

Calculations and statistics

The inhibitory response to each agonist drug concentration was calculated as the % decrease in spontaneous contraction amplitude. From each concentration-response curve, an EC_{50} value was determined as the concentration of agonist causing 50% of the maximum response to that agonist, and these values (when obtained in the absence of antagonist) were used to calculate pD_2 values ($-\log EC_{50}$). When antagonism appeared competitive the ratios of the EC_{50} values in the presence and absence of antagonist were calculated for each concentration of antagonist used. For each agonist, the data from six experiments were pooled to construct a Schild plot (Arunlakshana & Schild, 1959). The slope and x-intercept of the regression line were calculated by linear least squares regression analysis as described by Colton (1974). The x-intercept was taken as the pA_2 value if the slope of the regression line was not statistically significantly different from 1.

The significance of any deviation from 1 of the slopes of the Schild plots was determined by Student's *t* test. The statistical significance of the difference between original and subsequent

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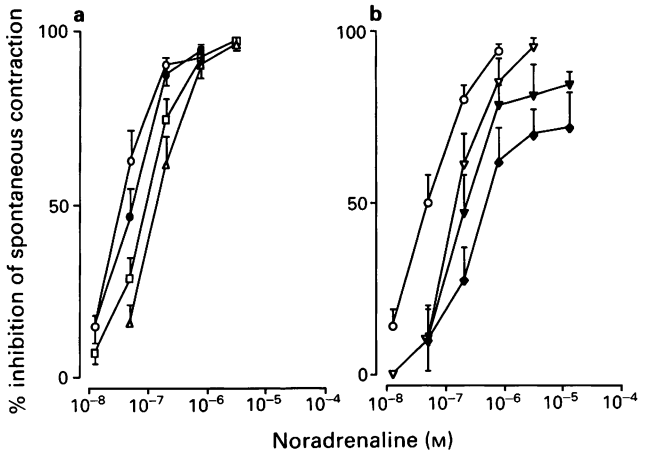

Figure 1 Inhibition of spontaneous activity in the rabbit jejunum. Concentration-response curves for noradrenaline in the absence (○) and presence of (a) propranolol (●, 34 nM; □, 340 nM; △, 3.4 μM), or (b) phentolamine (▽, 270 nM; ▼, 2.7 μM; ◆, 27 μM). Each point is the mean of 6 observations. Vertical lines show s.e.mean.

responses obtained in control tissues was tested by the Wilcoxon signed rank sum test. A difference was assumed to be significant when $P < 0.05$.

Results

Noradrenaline (13 nM–810 nM) inhibited spontaneous contractions of the rabbit jejunum in a concentration-dependent manner. The concentration-response curve for noradrenaline was shifted to the right by both propranolol (34 nM, 340 nM, 3.4 μM) and phentolamine (270 nM, 2.7 μM, 27 μM) (Figure 1). The highest concentration of phentolamine also suppressed the maximum response to the agonist.

Ritodrine (150 nM–4.9 μM) and salbutamol (840 nM–53 μM), like noradrenaline, inhibited spontaneous contraction of the rabbit jejunum in a concentration-dependent manner. The concentration-response curves for ritodrine and salbutamol were also shifted to the right by propranolol (340 nM, 1.1 μM, 3.4 μM, 10 μM) (Figure 2a and c). At a low concentration phentolamine (2.7 μM) had no significant ($P > 0.05$) effect (Figure 2b and d), but at a high concentration (27 μM) it suppressed the maximum responses to ritodrine and salbutamol.

The pD_2 values for noradrenaline, ritodrine and salbutamol are given in Table 1, together with Schild plot slopes and x-intercepts for propranolol antagonizing the action of these 3 agonists. The Schild plots are shown in Figure 3. In the cases of ritodrine and salbutamol the difference between the Schild plot slopes and 1 is not statistically significant, indicating competitive antagonism and thus allowing the x-intercepts for these agonists to be taken as the pA_2 values. However, with

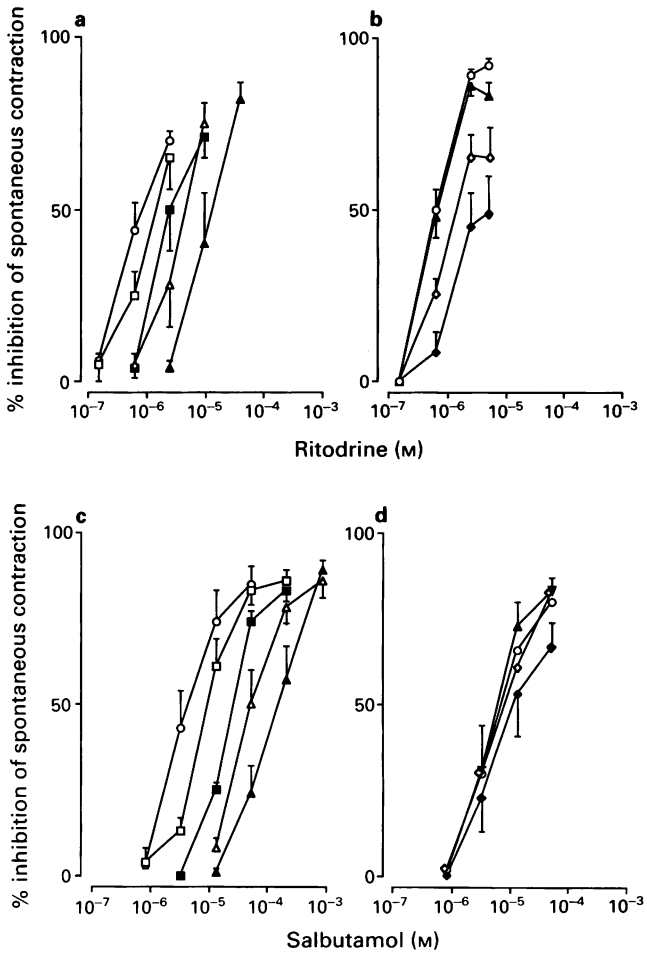

Figure 2 Inhibition of spontaneous activity in the rabbit jejunum. Concentration-response curves for ritodrine in the absence and presence of (a) propranolol and (b) phentolamine, and for salbutamol in the absence and presence of the same antagonists (c and d). (○) Indicates absence of antagonist. Propranolol concentrations used were: (□) 340 nM; (■) 1.1 μM; (△) 3.4 μM; (▲) 10 μM. Phentolamine concentrations used were: (▼) 2.7 μM; (◇) 8.8 μM; (◆) 27 μM. Each point is the mean of 6 observations. Vertical lines show s.e.mean. On two concentration-response curves in (d) s.e.mean values have been omitted for clarity.

noradrenaline the difference between the slope and 1 is highly significant ($P < 0.001$), so the antagonism is either non-competitive or not occurring at some of the receptors at which noradrenaline is acting.

In control tissues the difference between original and subsequent responses to agonist were not statistically significant ($n = 6$ for each agonist). In addition the effects of ritodrine

Table 1 Inhibition of spontaneous activity in the rabbit jejunum. pD_2 values, Schild plot slopes, x-intercepts and pA_2 values obtained with various agonists and propranolol

		Propranolol Schild plot	
Agonist	*pD_2 value*[a]	*Slope*[b]	*x-intercept or pA_2 value*
Noradrenaline	7.4 ± 0.09 (12)*	0.28 ± 0.13 (6,18)†	6.9‡
Ritodrine	6.2 ± 0.04 (12)	0.88 ± 0.11 (6,24)	6.4§
Salbutamol	5.3 ± 0.10 (12)	0.93 ± 0.16 (6,24)	6.6§

Values shown as mean ± s.e.mean[a] or ± s.e.[b].
* Number of experiments.
† Number of experiments, number of points on Schild plot.
‡ x-intercept (slope significantly different from 1, $P < 0.05$).
§ pA_2 value (slope not significantly different from 1, $P > 0.05$).

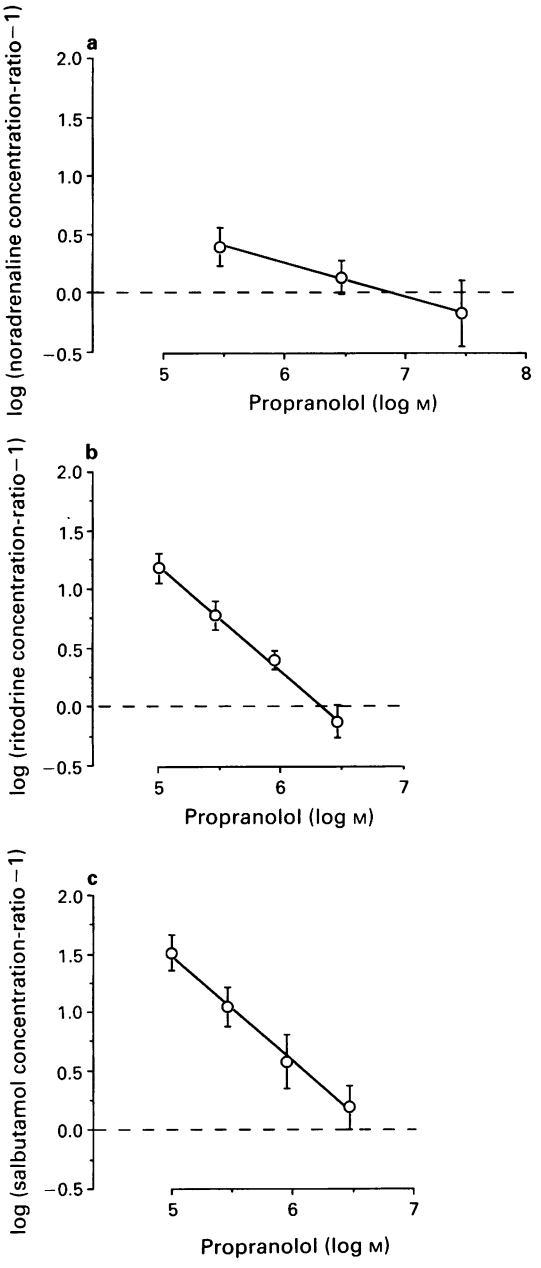

Figure 3 Schild plots (Arunlakshana & Schild, 1959) for propranolol antagonizing (a) noradrenaline, (b) ritodrine and (c) salbutamol in the rabbit jejunum. The regression lines shown were calculated by use of linear least squares regression analysis. Each point illustrated is the mean of 6 values. Vertical lines show s.e.mean.

(0.30 μM) or salbutamol (3.3 μM) were unaffected by the presence of yohimbine (2.6 μM), mepyramine (2.5 μM) or cimetidine (4.0 μM) ($n = 6$ for each agonist).

Discussion

The results are consistent with the existence in the rabbit jejunum of a β-adrenoceptor distinct from the well characterized β_1- or β_2-subtypes. Evidence for this can be drawn from both the pA_2 values obtained for propranolol and the relative potencies of salbutamol and ritodrine.

Established pA_2 values for propranolol acting at β_1- or β_2-adrenoceptors lie between 8.0 and 9.0 (Bristow *et al.*, 1970; Farmer & Coleman, 1970; Farmer & Levy, 1970; Lulich *et al.*, 1976), while the pA_2 value for propranolol acting against noradrenaline in the presence of phentolamine in the rabbit ileum has been determined as 9.8 (Wagner *et al.*, 1981). The considerable difference between these pA_2 values and those obtained in the present study (6.4 and 6.6 with ritodrine and salbutamol, respectively) indicates that in the rabbit jejunum propranolol antagonizes ritodrine and salbutamol at receptors different from conventional β_1- or β_2-adrenoceptors. As the slope of the Schild plots obtained with each agonist is close to 1 it is likely that both agonists are acting mainly at receptors where propranolol is a competitive antagonist. Furthermore, as the pA_2 values obtained with propranolol against ritodrine or salbutamol are similar both agonists are probably acting mainly at the same receptor.

These atypical adrenoceptors may co-exist with β_1-adrenoceptors identified in this tissue by Lands *et al.* (1967). It seems unlikely that the inhibition of spontaneous activity in the rabbit jejunum observed in our study is mediated by β_2-adrenoceptors because ritodrine was approximately 8 times as potent as salbutamol, as illustrated by a difference in pD_2 values for these agonists of just under 1 log unit, whereas ritodrine is less potent than salbutamol on conventional β_2-adrenoceptors in tissues such as the rat uterus or guinea-pig lung strip. For example, in uterus preparations from pregnant or ovariectomised rats, pD_2 values for ritodrine inhibiting the amplitude of longitudinal muscle contraction ranged from 7.24 (Ikeda & Tamaoki, 1984) to 7.3 (as calculated from the EC_{50} value, Izumi & Kishikawa, 1982), while corresponding values for salbutamol were in the range of 8.36 to 9.72 (Hollingsworth & Schnieden, 1973; Granger *et al.*, 1985). Likewise in the guinea-pig lung strip the pD_2 value for ritodrine-mediated relaxation was approximately 5.4 (as estimated from graphed data of Broadley *et al.*, 1986), while for salbutamol the pD_2 was approximately 7.0 (as estimated from graphed data of Schreurs *et al.*, 1980). These differences between relative potencies in tissues with conventional β_2-adrenoceptors compared with rabbit jejunum suggests that ritodrine and salbutamol are acting at receptors in the rabbit jejunum which are not typical β_2-adrenoceptors. However, the possibility that there is atypical coupling rather than an atypical receptor cannot be excluded.

To justify the designation of these receptors as a β-adrenoceptor subtype the possibility of these responses being mediated through a previously detected receptor not classified as a β-adrenoceptor needs to be excluded. α-Adrenoceptors have been shown to inhibit rabbit ileum contractile activity (Furchgott, 1960), but the present findings that only high concentrations of phentolamine (> 2.7 μM) antagonized the effects of ritodrine and salbutamol and that this antagonism was non-competitive are not consistent with blockade of conventional α_1-adrenoceptors. Moreover, although the responses to noradrenaline were antagonized competitively by low concentrations of phentolamine, at a high concentration the antagonism was of a non-competitive nature. The possibility that salbutamol and ritodrine act at α_2-adrenoceptors or H_1- or H_2-receptors can probably be excluded because yohimbine, mepyramine or cimetidine respectively at concentrations known to be effective at these receptors in other tissues (Arunlakshana & Schild, 1959; Brimblecombe *et al.*, 1975; Fuder *et al.*, 1983), did not alter the action of either adrenoceptor agonist in the rabbit jejunum.

There have been a number of reports of atypical β-adrenoceptors in various tissues. Directly relevant to the results given above is the atypical β-adrenoceptor which mediates lipolysis in both brown and white adipocytes (Harms *et al.*, 1974; Arch *et al.*, 1984; Wilson *et al.*, 1984; Grassby *et al.*, 1987). At this receptor the pA_2 values obtained for propranolol range from 6.2 to 6.84 (Harms *et al.*, 1974; Wilson *et al.*, 1984), while the pD_2 value for ritodrine is approximately 6.0 (as estimated from the graphed results of Grassby *et al.*, 1987) and the pD_2 values for salbutamol range from 4.9 to 5.6 (as calculated from EC_{50} values obtained by Wilson *et al.*, 1984 and Arch *et al.*, 1984). These values correspond fairly closely to those described here for the atypical β-adrenoceptor in the rabbit jejunum. Coleman *et al.* (1987) demonstrated, in the

guinea-pig gastric fundus, an atypical β-adrenoceptor which mediated relaxation and which appears to be similar in its sensitivity to propranolol and salbutamol to the rabbit jejunal β-adrenoceptor described here. Thus, at least two other tissues may possess atypical β-adrenoceptors which are similar in agonist and antagonist affinities to those atypical β-adrenoceptors described here. BRL 35135A is a potent and selective agonist at the atypical β-adrenoceptors in both these tissues (Wilson *et al.*, 1984; Colman *et al.*, 1987), but the action of this agent in the rabbit jejunum remains to be tested. An atypical adrenoceptor which inhibits longitudinal muscle tension development in the guinea-pig ileum has also been described (Bond & Clarke, 1988). However, this adrenoceptor, unlike that described here, is resistant to blockade by propranolol. There is also evidence for an atypical heart β-adrenoceptor in a number of different species (Kaumann, 1989).

In conclusion, the pA_2 values for propranolol acting against ritodrine and salbutamol, and the relative potencies of these agonists, differ in the rabbit jejunum from values for tissues containing typical β_1- or β_2-adrenoceptor subtypes. This constitutes provisional evidence for a β-adrenoceptor subtype not previously described in the rabbit jejunum but which appears to resemble the adrenoceptors of adipocytes and guinea-pig gastric fundus. However, further experimentation with a broader range of antagonists is required.

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