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Agonist and antagonist characterization of a putative adrenoceptor with distinct pharmacological properties from the α- and β-subtypes

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1 Experiments were done to characterize a putative adrenoceptor which functions to inhibit longitudinal muscle tension development in the guinea-pig ileum. Several phenylethylamine based agonists were investigated: BRL 37344, (−)-isoprenaline, (+)-isoprenaline, noradrenaline, adrenaline, and fenoterol. Propranolol and nadolol were tested as antagonists. Agonist-induced inhibition of the contractile response to histamine was measured under equilibrium conditions with α-adrenoceptors and muscarinic cholinoceptors inhibited.

2 Inhibitory responses were obtained to (−)-isoprenaline and BRL 37344 that were resistant to β-adrenoceptor blockade with propranolol (5 μM) and nadolol (10 μM). These resistant responses were antagonized by much higher concentrations of nadolol (30 to 1000 μM) yielding apparent pA_2 values for nadolol of 4.31 with (−)-isoprenaline as the agonist, and 4.68 with BRL 37344 as the agonist. Similar apparent pA_2 values for nadolol at the putative adrenoceptor were obtained with noradrenaline (4.79), adrenaline (4.68), and fenoterol (4.38).

3 The order and relative potency of agonists at the putative adrenoceptor was: BRL 37344 (20) > (−)-isoprenaline (8) > noradrenaline (1) > adrenaline (0.5) > fenoterol (0.35) > (+)-isoprenaline (0.27).

4 The resistance to blockade by propranolol (5 μM), the low affinity of nadolol, and the order and relative potency of agonists, suggest the presence of an adrenoceptor with distinct pharmacological characteristics from currently defined α- and β-adrenoceptors.

Introduction

Although adrenoceptors have been subdivided into the α- and β-subtypes (Ahlquist, 1948; Lands *et al.*, 1967a,b; Langer, 1974; Berthelsen & Pettinger, 1977) their classification and characterization is still incomplete. Based upon quantitative pharmacological experiments we have postulated the existence of an adrenoceptor distinct from defined α- and β-subtypes (Bond *et al.*, 1986a,b; Bond & Clarke, 1987a). This putative adrenoceptor functions to inhibit tension development in the guinea-pig ileum and is activated by noradrenaline and (−)-isoprenaline (isoprenaline) but not by clonidine, UK14,304-18 (5-bromo-6-[2-imidazolin-2-yl-amino]-quinoxaline), and St 587 [2-(2-chloro-5-trifluoro-methyl-phenylimino)imidazolidine]. The putative adrenoceptor is resistant to blockade by α-adrenoceptor antagonists (phentolamine, rauwolscine, idazoxan, benextramine) and β-adrenoceptor antagonists (propranolol and nadolol) at concentrations that saturate their respective receptor sites (at least 100 times their equilibrium dissociation constants).

The object of the present experiments was to define better the putative adrenoceptor. This task has been approached by use of several phenylethylamine based agonists and the nonselective β-adrenoceptor antagonist, nadolol (Lee *et al.*, 1975). We now show that nadolol exhibits a low affinity for the putative adrenoceptor and that BRL 37344 (Arch *et al.*, 1984) acts as the most potent agonist yet identified at the proposed site.

Preliminary accounts of this work were given at the Xth International Congress of Pharmacology (Bond & Clarke, 1987b) and to the British Pharmacological Society (Bond *et al.*, 1988).

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Methods

Tissue preparation

Male albino guinea-pigs (Duncan Hartley strain) weighing between 300 and 500 g were pretreated with reserpine (5 mg kg^{-1}, i.p. for 18 h) and were killed by decapitation. The distal ileum was removed and the first 10 cm, proximal to the ileo-caecal junction, was discarded. After carefully washing out the luminal contents, segments of about 3 cm in length, were suspended in a 20 ml organ bath under an initial tension of 1.0 g in Krebs solution at 37°C. The Krebs solution contained (mM): NaCl 118, $CaCl_2$ 2.6, KCl 4.9, $NaHCO_3$ 25, NaH_2PO_4 1, $MgSO_4$ 1.2, glucose 11.7, and ascorbic acid 0.11 to offset autooxidation of catecholamines; it was bubbled continuously with 5% CO_2 and 95% O_2 to give a pH of 7.42. In all experiments cocaine (30 μM), corticosterone (30 μM), phentolamine (3 μM), and atropine (1 μM) were present in the Krebs solution to inhibit neuronal uptake, extraneuronal uptake, α-adrenoceptors, and muscarinic cholinoceptors respectively (Bond *et al.*, 1986a,b; Bond & Clarke, 1987a). The concentrations of phentolamine and atropine selected are at least 100 times their equilibrium dissociation constants for α-adrenoceptors and muscarinic receptors respectively (Kenakin, 1987; Clague *et al.*, 1985). A tissue equilibrium time of 45 min was allowed before starting experiments.

Concentration-effect curves

The inhibitory activity of agonists was determined by measuring the reduction in the contractile response to a submaximal concentration of histamine (0.5 μM). The contractile response of the ileum to the first exposure to histamine was variable and was not measured. Subsequent contractions to histamine showed little variation (Figure 1) when elicited at 5 min intervals (responses numbered 1 to 8) with 5 washes between each response, and at 10 min intervals (responses numbered 8 to 10) with 10 washes between each response. The first of these responses (response 1, Figure 1) was set as 100% and all subsequent responses were expressed as a percentage change. Increasing concentrations of agonists were added to ileal segments 1 to 3 min before the addition of histamine and were retained in the organ bath during the response to histamine. The contact time for achieving steady-state responses to agonists was determined from pilot experiments. Antagonists were added to the reservoir of Krebs solution and were allowed to equilibrate with the tissue for 30 min before the first exposure of the tissue to histamine.

In order to avoid desensitization, only one concentration-effect curve to an agonist was constructed per segment of ileum. Concentration-effect curves in the presence and absence of antagonists were done on adjacent segments of ileum.

pA_2 values

Some pA_2 values were determined by taking the negative logarithm of the antagonist equilibrium dissociation constant (K_B), as described by Furchgott (1972).

$$K_B = \frac{\text{Antagonist concentration } (M)}{\text{Agonist concentration ratio} - 1}$$

Other pA_2 values were determined from Arunlakshana & Schild (1959) plots. Confidence limits (CL at 95% probability) were computed for the slopes of the Arunlakshana & Schild (1959) plots using Stat View 512+ (Brain Power Inc., 24009 Ventura Boulevard, Calabasas, CA 91302, U.S.A.). All agonist concentration-ratios (CR) quoted were measured at the IC_{30} point on the agonist concentration-effect curves because of the parallel nature of the curves at this point. The IC_{30} is the concentration of agonist causing 30% inhibition of the response to histamine.

Drugs

The drugs listed below were prepared in distilled water with the exception of corticosterone which was dissolved in dimethylsulphoxide and reserpine which was dissolved in 20% w/v ascorbic acid solution and then titrated to pH 5.5 with sodium hydroxide. The drugs were obtained from the following sources: (−)-isoprenaline hydrochloride, (+)-isoprenaline hydrochloride, (±)-propranolol hydrochloride, corticosterone, cocaine hydrochloride, ascorbic acid, reserpine, angiotensin II, carbachol chloride, (−)-noradrenaline bitartrate, and adrenaline bitartrate were purchased from Sigma, St Louis, MO, U.S.A.; atropine sulphate monohydrate from Calbiochem, San Diego, CA, U.S.A.; histamine dihydrochloride from Calbiochem-Behring, LaJolla, CA, U.S.A.; phentolamine mesylate from Ciba-Geigy, Summit, NJ, U.S.A.; fenoterol was obtained as a gift from Boehringer Ingelheim Ltd, Ridgefield, CT, U.S.A. Nadolol was kindly supplied by Dr Gunnar Aberg, E. R. Squibb & Sons, Inc, Princeton, NJ, U.S.A. and BRL 37344, sodium-4-[2-[2-hydroxy-2-(3-chlorophenyl)ethylamino]propyl]phenoxyacetate sesquihydrate (RR.SS diestereo-isomer), was kindly supplied by Dr Jonathan R. S. Arch, Beecham Pharmaceuticals Research Division, Great Burgh, Epsom, Surrey, England.

Results

Experiments were designed to evaluate agonists in the absence and presence of β-adrenoceptor blockade. The latter experiments were performed in the presence of propranolol, 5 μM. The rationale for selecting the propranolol concentration was based upon findings reported previously (Bond & Clarke, 1987a). In these experiments, the cholinergically mediated 'twitch' response to transmural electrical stimulation of guinea-pig ileum was recorded and the inhibitory response to isoprenaline, and its interaction with propranolol (0.1, 1, and 5 μM), was studied. All three concentrations of propranolol caused about the same dextral shift (1 to 1.2 log units) in the concentration-effect curve to isoprenaline, demonstrating that propranolol, at 5 μM, saturates β-adrenoceptors. In addition, 5 μM propranolol is the highest concentration that can be used reliably without evoking smooth muscle relaxation (Figure 6).

Figure 1 illustrates a single experiment in which the interaction of isoprenaline and propranolol (5 μM) was studied with histamine as the spasmogen. Panel (a) shows that contractile responses to histamine (0.5 μM) remained constant over a 55 min time period. Constant responses to histamine were also obtained in segments of ileum that had been preincubated for 30 min with propranolol (5 μM) (data not shown). Panel (b) shows the effect of isoprenaline (0.001 to 100 μM) on the response to histamine. Isoprenaline was added to the bath 1 min before histamine responses numbered 3 to 8 and was present in the bath during the histamine responses. As shown, isoprenaline inhibited the response to histamine compared with the initial control response (response 1). The IC_{30} for isoprenaline was 0.009 μM. Washing the tissue (every min for 10 min) restored the histamine response to 50% (response 9) of control and a further 10 washes restored the response to histamine to 78% (response 10) of control. The inhibition by isoprenaline was not singular to the use of histamine

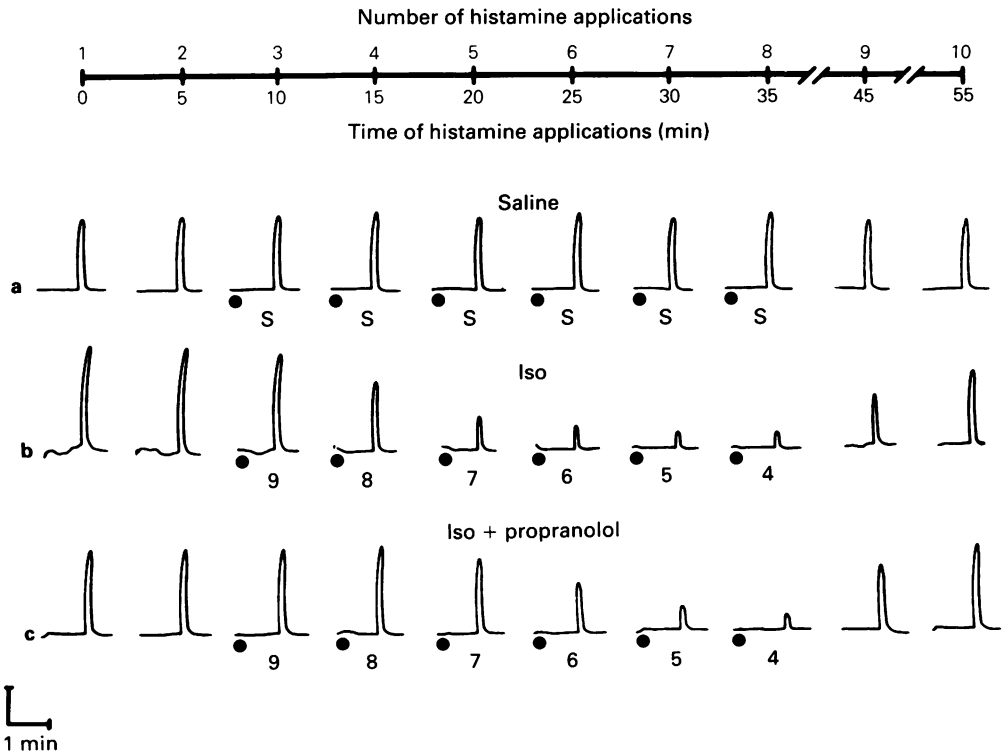

Figure 1 Effect of isoprenaline (Iso) on the contractile response to histamine (0.5 μM) in isolated segments of guinea-pig ileum: interaction with propranolol (5 μM). The number of histamine applications and the time at which they were made is given at the top of the Figure. In (a), control responses to histamine, the effect of saline (S, responses 3 to 8) and recovery from S (responses 9 to 10). In (b), Iso (responses 3 to 8) and recovery from Iso (responses 9 and 10). In (c), preincubated with propranolol (5 μM); Iso (responses 3 to 8) and recovery from Iso (responses 9 and 10). Shown are concentrations of Iso (−log M). For further details see Methods.

as the spasmogen because similar results were obtained with angiotensin II or carbachol (0.5 mM and 0.5 μM respectively; data not shown) and the 'twitch' response to transmural electrical stimulation of the ileum (Bond & Clarke, 1987a). Panel (c) shows the effect of propranolol (5 μM) on the inhibitory response to isoprenaline. The propranolol was preincubated with the tissue for 30 min before the start of the experiment. Compared with the control response to histamine (response 1), isoprenaline, in the presence of propranolol, still caused inhibition of the histamine contractions. The IC_{30} for isoprenaline in the presence of propranolol was 0.4 μM. The response to histamine returned to 70% (response 9) of control after 10 washes and 100% (response 10) after a further 10 washes. Thus, propranolol, in this single experiment, caused about a 40 fold shift in the concentration-effect curve to isoprenaline.

Figure 2 gives mean data for isoprenaline and its interaction with propranolol (5 μM) obtained in a previous set of experiments. In these experiments, propranolol caused a parallel mean dextral shift of 16 fold in the concentration-effect curve to isoprenaline with no change in the maximum response. A similar shift, 15 fold, was obtained with propranolol (5 μM) in ilea taken from guinea-pigs ($n = 6$) which had not been treated with reserpine (data not shown). These shifts, and the shift illustrated in Figure 1, are considerably less than expected for competitive antagonism at β-adrenoceptors by propranolol. For competitive antagonism between isoprenaline and propranolol, a dextral shift of 1,582 would be required (see theoretical curve in Figure 2), assuming a pA_2 value of 8.5 for propranolol at β-adrenoceptors (Farmer & Levy, 1970; Harms, 1976; O'Donnell & Wanstall, 1979; Wilson *et al.*, 1984). The failure of propranolol to interact competitively with isoprenaline is not due to α-adrenoceptor agonism by isoprenaline or to agonism at dopamine receptors because the experiments were done in the presence of a saturating concentration of phentolamine (3 μM) and inhibitory receptors for dopamine are absent from the guinea-pig ileum (Görich *et al.*, 1981). Thus, as reported previously and as is evident from the present study, isoprenaline exerts an action at a site (putative adrenoceptor) that is distinct from currently defined adrenoceptors.

Results from experiments in which the interaction of propranolol (5 μM) was studied versus isoprenaline, (+)-isoprenaline, and several other sympathomimetic amines, are shown in Table 1. Propranolol caused parallel dextral shifts in the concentration-effect curves which ranged from about 4 to 38 fold with little change in maximal response. All shifts with propranolol are considerably less than predicted for a competitive interaction at β-adrenoceptors (see above). It is important to note that the putative

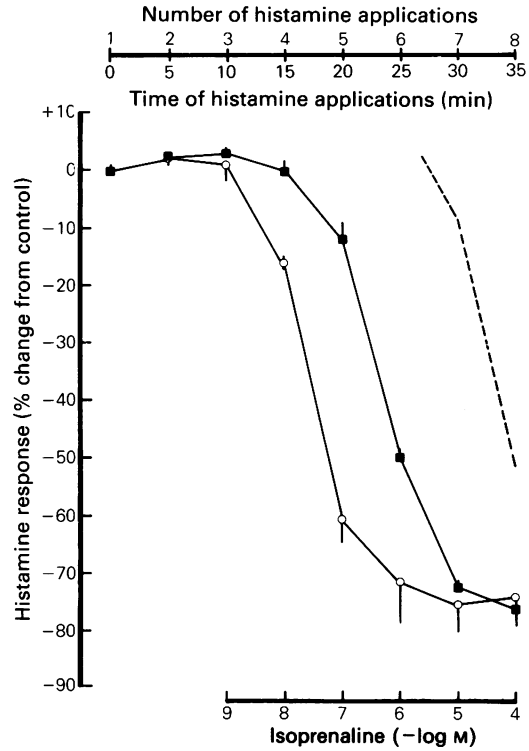

Figure 2 Inhibitory effect of isoprenaline (Iso) on the contractile response to histamine (0.5 μM) in isolated segments of guinea-pig ileum: interaction with propranolol (5 μM). The number of histamine applications and the time at which they were made is given at the top of the Figure: (○) control ($n = 6$); (■) effect of propranolol ($n = 6$); (----) theoretical position for the concentration effect curve to Iso in the presence of propranolol (5 μM) assuming only β-adrenoceptors and a pA_2 value of 8.5 for propranolol. Each point is the mean percentage inhibition obtained from 6 guinea-pigs; vertical lines show s.e. of the ratio.

adrenoceptor identified by isoprenaline in the presence of propranolol recognizes optical isomers. The isomeric ratio, (+)-isoprenaline/isoprenaline, before and after propranolol, was 16 and 29 respectively. Furthermore, the dextral shift with BRL 37344 was small (4 fold), indicating that the bulk of its inhibitory action toward histamine cannot be attributed to β-adrenoceptor agonism.

From the relative potencies and adrenoceptor preferences of the sympathomimetic amines listed in Table 1 it was reasoned that the putative adrenoceptor may be more 'β-like' than 'α-like'. We have shown previously (Bond *et al.*, 1986a,b) that selective α-adrenoceptor agonists are without activity at the putative adrenoceptor. Therefore, it was hypothesized that a β-adrenoceptor antagonist might exert a

Table 1 Relative potencies and effect of propranolol (5 μM) on the inhibitory response to phenylethylamine based agonists in isolated segments of guinea-pig ileum

Agonist	IC_{30} (without propranolol)	r	α	IC_{30} (with propranolol)	r	α	*CR*
(−)-Isoprenaline	0.020 ± 0.0016	3	0.76 ± 0.06	0.30 ± 0.017	8	0.76 ± 0.04	16
BRL 37344	0.027 ± 0.0019	2.2	0.80 ± 0.06	0.11 ± 0.009	20	0.72 ± 0.06	4.1
Noradrenaline	0.06 ± 0.003	1	0.70 ± 0.04	2.30 ± 0.138	1	0.55 ± 0.04	38
Adrenaline	0.28 ± 0.020	0.21	0.75 ± 0.09	4.80 ± 0.384	0.5	0.55 ± 0.06	17
(+)-Isoprenaline	0.31 ± 0.022	0.19	0.76 ± 0.08	8.60 ± 0.606	0.27	0.64 ± 0.06	28
Fenoterol	0.36 ± 0.032	0.17	0.80 ± 0.09	6.80 ± 0.706	0.35	0.65 ± 0.07	19

IC_{30}, the concentration (μM) of agonist producing 30% inhibition of the contractile response to histamine 0.5 μM; r, relative potency, noradrenaline = 1; α, intrinsic activity; CR, concentration ratio (before and after propranolol) measured at the IC_{30} values. Shown are mean values obtained from 4 to 6 guinea-pigs.

low affinity for the proposed site. In this regard nadolol was selected because it is devoid of local anaesthetic activity (Lee *et al.*, 1975) thus permitting the use of high concentrations. Nadolol was studied over a concentration range of 10,000 fold (0.1 μM to 1 mM) and did not affect the reproducibility or magnitude of the responses to histamine, except at 1 mM, where it caused a 20–30% reduction in the contractile response to histamine. Figure 3 illustrates the results obtained with nadolol versus isoprenaline as the agonist. Nadolol, 0.1 μM (curve 2) and 1 μM (curve 3) produced parallel mean dextral shifts in the concentration-effect curve to isoprenaline of 5.0 and 42.7 fold respectively, with little or no change in the maximum responses. However, a 10 fold increase in the concentration of nadolol, to 10 μM (curve 4), failed to produce a further dextral shift. In the presence of 10 μM nadolol, the mean dextral shift from control was still only 40.8 fold indicating that β-adrenoceptors were fully blocked. At this point, further increases in the concentration of nadolol once again evoked shifts in the concentration-effect curve to isoprenaline. Mean shifts of 157 and 1996 from control were obtained with nadolol 100 μM (curve 5) and 1 mM (curve 6) respectively. These data are plotted as an Arunlakshana & Schild (1959) plot in Figure 4a. The first phase of the plot gives an apparent pA_2 of 7.56 for nadolol with a slope of 1.02 (95% CL 0.74–1.29). The second phase of the plot has been redrawn and is represented in Figure 4b. For this plot, the mean IC_{30} obtained from curves 3 and 4 in Figure 3 was used as the control point from which the shifts for nadolol 100 μM (curve 5) and 1 mM (curve 6) were calculated. From Figure 4b, an apparent pA_2 of 4.31 with a slope of 1.25 (95% CL 0.96–1.54) was obtained.

In another set of experiments, concentration-effect curves to isoprenaline, noradrenaline, adrenaline, and fenoterol were constructed in the presence of propranolol, 5 μM, to saturate β-adrenoceptors and, in a parallel set of experiments, in the presence of nadolol (1 mM). The difference between IC_{30} values

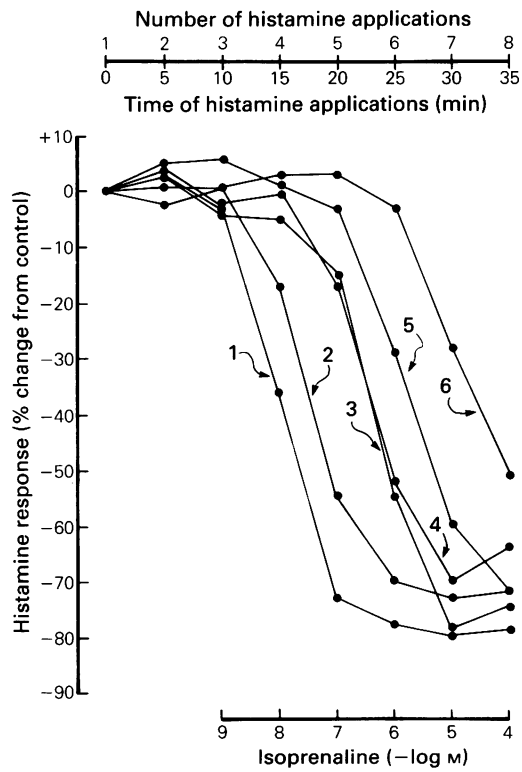

Figure 3 Inhibitory effect of isoprenaline on the contractile response to histamine (0.5 μM) in isolated segments of guinea-pig ileum: interaction with nadolol. The number of histamine applications and the time at which they were made is given at the top of the figure. Curve 1 represents control values (n = 25). Curve 2 (n = 5), curve 3 (n = 5), curve 4 (n = 5), curve 5 (n = 6), and curve 6 (n = 4) were done in the presence of nadolol 0.1, 1.0, 10, 100, and 1000 μM respectively. Each point is the mean percentage inhibition obtained from the number of guinea-pigs given by the value of n. (Standard errors of the ratios were less than 6% of mean values and are not illustrated.)

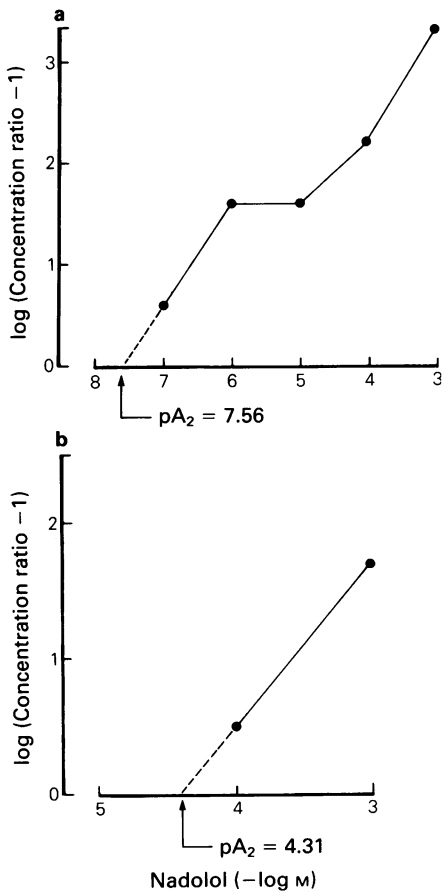

Figure 4 Arunlakshana & Schild (1959) plots for nadolol versus isoprenaline: In (a), concentration-ratios were measured from the IC_{30} of curve 1 in Figure 3. In (b), concentration-ratios were measured from the mean of the IC_{30} values obtained from curves 3 and 4 in Figure 3.

for each agonist under the two experimental conditions was used to calculate apparent pA_2 values for nadolol according to the method of Furchgott (1972). The following pA_2 values (mean ± s.e.) were obtained: isoprenaline 4.47 ± 0.13 ($n = 4$), noradrenaline 4.49 ± 0.10 ($n = 6$), adrenaline 4.68 ± 0.14 ($n = 3$), and fenoterol 4.38 ± 0.10 ($n = 4$). These pA_2 values for nadolol at the putative adrenoceptor agree well with those derived from Arunlakshana & Schild (1959) plots (Figures 4b and 8b), in which nadolol (1 to 10 μM), instead of propranolol (5 μM), was used to establish control conditions. However, the possibility exists that the 20–30% reduction in the contractile response to histamine, evoked by 1 mM nadolol, influenced the determination of pA_2 values at the putative adrenoceptor. In this regard, experiments revealed that a similar percentage reduction in the contractile response to histamine, elicited by using a lower concentration of histamine (0.25 μM), did not alter the IC_{30} of isoprenaline, either in the absence or presence of propranolol, 5 μM (data not shown). Other experiments showed that nadolol, 1 mM, failed to antagonize the inhibitory actions of papaverine toward histamine induced contractions (Figure 5). Instead, the response to 10 μM papaverine was increased by nadolol. Finally, the nonspecific inhibitory response to high concentrations of propranolol in guinea-pig ileum (beyond 5 μM; Grassby & Broadley, 1987) was not affected by nadolol, 1 mM (Figure 6).

The results described above support the notion of a novel adrenoceptor in guinea-pig ileum with a low affinity for nadolol. An adrenoceptor exhibiting a low affinity for β-adrenoceptor antagonists has been identified on fat cells and is stimulated selectively by the phenylethylamine analogue, BRL 37344 (Arch *et al.*, 1984; Wilson *et al.*, 1984). Figure 7 shows that BRL 37344 inhibited the contractile response to histamine in the guinea-pig ileum and that this effect

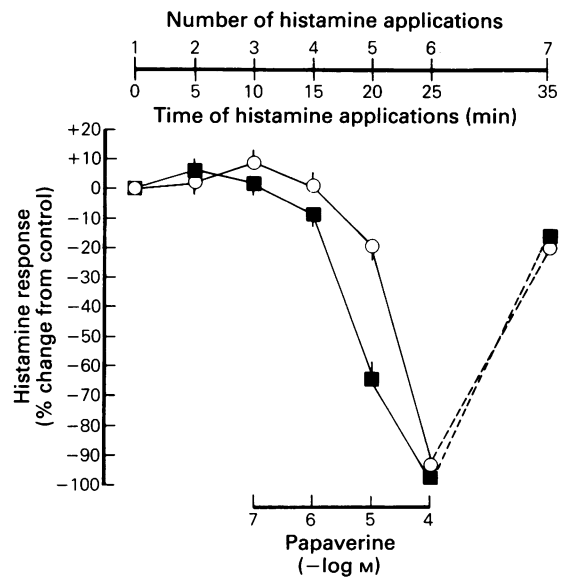

Figure 5 Inhibitory effect of papaverine on the contractile response to histamine (0.5 μM) in isolated segments of guinea-pig ileum: interaction with nadolol (1 mM). The number of histamine applications and the time at which they were made is given at the top of figure. (○) control ($n = 5$); (■) effect of nadolol ($n = 5$). Response 7 shows the recovery of the response to histamine following 10 washes made between 25 and 35 min. Each point is the mean percentage inhibition obtained from 5 guinea-pigs; vertical lines show s.e. of the ratio.

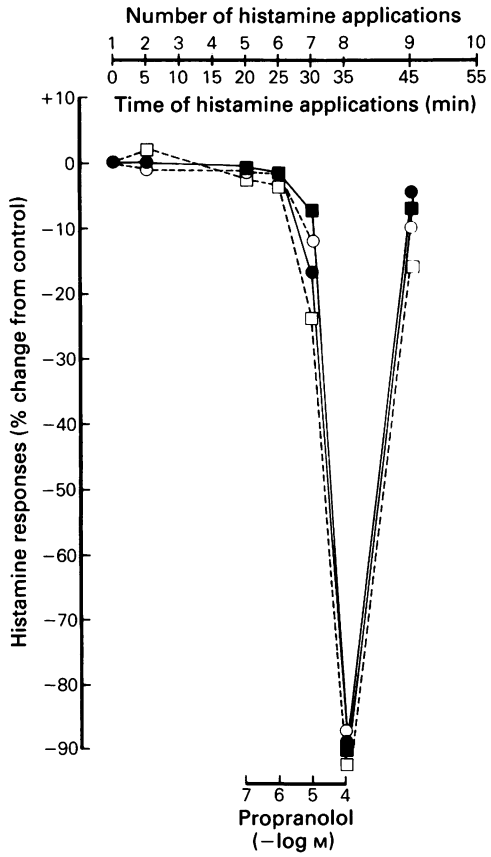

Figure 6 Inhibitory effect of propranolol on the contractile response to histamine (0.5 μM) in isolated segments of guinea-pig ileum: interaction with nadolol (1 mM). The number of histamine applications and the time at which they were made is given at the top of the figure. (○ and □) control responses to (+)-propranolol ($n = 4$) and (−)-propranolol ($n = 4$); (●) and (■) effect of nadolol on the responses to (−)-propranolol ($n = 4$) and (+)-propranolol ($n = 4$). Response 9 shows the recovery of the response to histamine following 10 washes made between 35 and 45 min. Each point is the mean percentage inhibition obtained from 4 guinea-pigs. (Standard errors of the ratios were less than 5% of mean values and are not illustrated.)

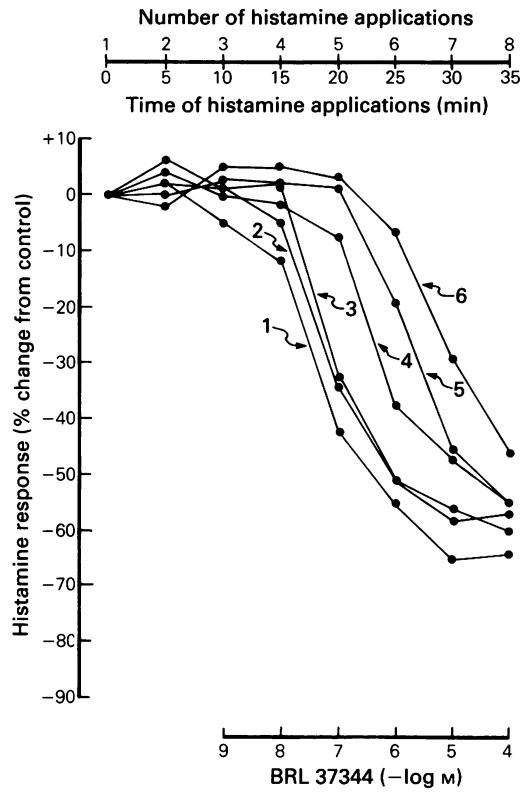

Figure 7 Inhibitory effect of BRL 37344 on the contractile response to histamine (0.5 μM) in isolated segments of guinea-pig ileum: interaction with nadolol. The number of histamine applications and the time at which they were made is given at the top of the figure. Curve 1 represents control values ($n = 21$). Curve 2 ($n = 4$), curve 3 ($n = 4$), curve 4 ($n = 5$), curve 5 ($n = 4$), and curve 6 ($n = 4$) were done in the presence of nadolol 3, 10, 100, 300, and 1000 μM respectively. Each point is the mean percentage inhibition obtained with the number of guinea-pigs given by the value of n. (Standard errors were less than 8% of mean values and are not illustrated.)

was antagonized by nadolol. Nadolol (3 and 10 μM) caused parallel mean dextral shifts of 2.2 and 2.3 respectively whereas concentrations of 100 μM, 300 μM, and 1 mM nadolol caused concentration-related shifts of 18, 59, and 252.2 respectively. These data are plotted as an Arunlakshana & Schild (1959) plot in Figure 8a. The second phase of the plot has been redrawn and is represented in Figure 8b. The mean IC_{30} obtained from curves 2 and 3 in Figure 7, was used to calculate the shifts for nadolol at 100 μM, 300 μM, and 1 mM. From Figure 8b, an apparent pA_2 of 4.68 with a slope of 1.20 (95% CL 0.99–1.41) was obtained.

Finally, it is important to note that the mean IC_{30} for BRL 37344 in the presence of nadolol (3 and 10 μM), is 0.079 μM (Figure 7), whereas the mean IC_{30} for isoprenaline in the presence of nadolol, (1 and 10 μM) is 0.224 μM (Figure 3). Thus, BRL 37344 is 2.8 times more potent at the putative adrenoceptor than isoprenaline. A similar potency ratio for the two agonists at the putative adrenoceptor was obtained with propranolol (Table 1).

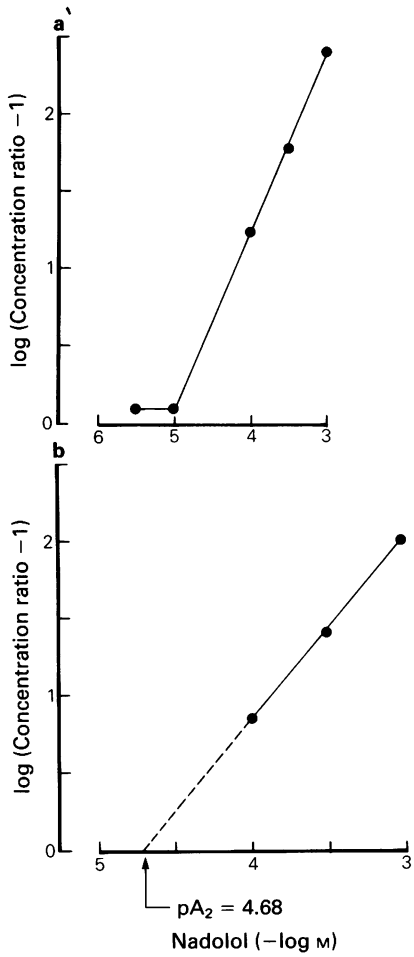

Figure 8 Arunlakshana & Schild (1959) plots for nadolol versus BRL 37344. In (a), concentration-ratios were measured from the IC_{30} of curve 1 in Figure 6. In (b), concentration-ratios were measured from the mean of the IC_{30} values obtained from curves 2 and 3 in Figure 7.

Discussion

The present findings with agonist and antagonist probes have confirmed that sympathomimetic amines of the phenylethylamine class can evoke an inhibitory response in the guinea-pig ileum which is not mediated via currently defined α- and β-adrenoceptors (Bond *et al.*, 1986a,b; Bond & Clarke, 1987a). However, it is suggested that the response is receptor-mediated because of (1) the concentration-dependence of the response; (2) the recognition of optical isomers of isoprenaline (this study) and noradrenaline (Bond *et al.*, 1986b); (3) blockade of the putative receptor by nadolol.

The presence of an undefined adrenoceptor in the guinea-pig ileum is evident from the failure of propranolol and nadolol to interact competitively with β-adrenoceptor agonists even though α-adrenoceptors were saturated with phentolamine (3 μM) and equilibrium conditions prevailed (depletion of endogenous monoamines with reserpine and the addition of ascorbic acid, cocaine, and corticosterone to the Krebs solution). For competition at a single population of β-adrenoceptors, propranolol, at 5 μM, should have shifted the concentration-effect curves to isoprenaline, noradrenaline, adrenaline, fenoterol, and BRL 37344 by about 1500 fold, assuming a pA_2 value for propranolol at β-adrenoceptors of 8.5 (Farmer & Levy, 1970; Harms, 1976; O'Donnell & Wanstall, 1979; Wilson *et al.*, 1984). Instead, limited shifts of 4 to 40 fold were obtained (Table 1). These data indicate the presence of another receptor site for which agonists exhibit a lower potency (4 to 40 fold less than at the β-adrenoceptor) and for which propranolol (5 μM) lacks affinity. A similar conclusion may be drawn from experiments with nadolol. With isoprenaline as the agonist, nadolol, 0.1 μM and 1.0 μM, interacted in a competitive manner but the 10 μM concentration of nadolol deviated clearly from competitive kinetics (Figures 3 and 4). Both the 1.0 μM and 10 μM concentrations of nadolol caused the same dextral shift (about 40 fold) in the concentration-effect curves to isoprenaline, indicating that β-adrenoceptors were saturated. However, further increases in the concentration of nadolol again evoked dextral shifts in the concentration-effect curve to isoprenaline. Thus, as with propranolol, a putative adrenoceptor for isoprenaline, resistant to α- and β-adrenoceptor blockade, was disclosed. Similarly, nadolol clearly distinguished two distinct effector receptor sites with BRL 37344 as the agonst (Figures 7 and 8).

Nadolol exhibited a low affinity for the putative adrenoceptor and apparent pA_2 values of 4.31 (with isoprenaline as the agonist) and 4.68 (with BRL 37344 as the agonist) were derived from Figures 4c and 8b. These values are about 3 orders of magnitude less than the pA_2 for nadolol at β-adrenoceptors. In this regard, Lee *et al.* (1975) reported a pA_2 of 7.7 for nadolol at β-adrenoceptors and an apparent pA_2 of 7.56 was found in the present study (Figure 4a). Whereas the slope of the Arunlakshana & Schild (1959) plot was nearly unity (1.02) for nadolol at β-adrenoceptors, slopes of 1.25 (with isoprenaline as the agonist) and 1.20 (with BRL 37344 as the agonist) were obtained with nadolol at the putative adrenoceptor. Such steep slopes, although not significantly different from 1 (95% CL), indicate the possible involvement of mechanisms

other than simple competition. Steep Arunlakshana & Schild (1959) plots for competititve antagonists may result because of progressive saturation (with increasing concentrations of the antagonist) of uptake or metabolizing mechanisms for the antagonist (Kenakin & Beek, 1987) or because of a diminution in the slopes of agonist concentration-effect curves when receptor reserve is limited or absent (Rang, 1966). Functional antagonism, in conjunction with receptor blockade, will likewise result in steep Arunlakshana & Schild (1959) plots (Hughes & Mackay, 1985). Nonspecific effects of nadolol may also be involved. Nadolol (1 mM) reduced responses to histamine by 20–30%. However, when this effect was mimicked experimentally, by reducing the histamine concentration to 0.25 μM, the IC_{30} for isoprenaline was not altered. Thus, in itself, a 20–30% reduction in the response to histamine may not be a confounding factor. On the other hand, nadolol (1 mM) increased responses to the 10 μM concentration of papaverine (Figure 5) but failed to influence the nonspecific relaxant responses (Grassby & Broadley, 1987) to high concentrations of propranolol (Figure 6). In view of the potentiating action of nadolol toward papaverine (10 μM), it would be prudent to regard the pA_2 values for nadolol at the putative adrenoceptor to be at best approximate. However, it is noteworthy that the range of all experimentally determined pA_2 values at this site is only 0.48 log units, despite the low affinity of nadolol for the putative adrenoceptor and the use of 5 different agonists. This close concordance, along with the use of multiple agonist probes, enhances confidence that a single site of action is involved. Finally, because the slopes of the Arunlakshana & Schild (1959) plots for nadolol and BRL 37344 are not significantly different from 1, refined estimates of pA_2 values for nadolol can be calculated by constraining the slopes to 1 (Stone & Angus, 1978). Employing this constraint, the following pA_2 values for nadolol were obtained: 7.57 (versus isoprenaline at β-adrenoceptors); 4.51 (versus isoprenaline at the putative adrenoceptor); 4.92 (versus BRL 37344 at the putative adrenoceptor).

The guinea-pig ileum is thought to contain postjunctional β_1-adrenoceptors (Mian *et al.*, 1984; Grassby & Broadley, 1984) and the relative potency of isoprenaline, noradrenaline, adrenaline, and fenoterol given in Table 1 would generally support this view. However, the apparently high potency of BRL 37344 relative to isoprenaline at β-adrenoceptors is an unusual finding (Arch *et al.*, 1984; Wilson *et al.*, 1984). This may be explained by the high potency of BRL 37344 at the putative adrenoceptor. The small shift in the concentration-effect curve to BRL 37344 by propranolol (5 μM) or nadolol (3 and 10 μM) demonstrates that the bulk of the concentration-effect curve to BRL 37344 originates from the putative adrenoceptor in the guinea-pig ileum rather than β_1-adrenoceptors. BRL 37344 has also been reported to relax guinea-pig stomach fundus with about equal potency to isoprenaline (Coleman *et al.*, 1987). Furthermore, the relaxant responses to BRL 37344 were only shifted 5 fold by 10 μM propranolol (Coleman *et al.*, 1987). Thus, the putative adrenoceptor recognizes 'classical' β-adrenoceptor agonists (isoprenaline, noradrenaline, adrenaline, fenoterol) with about the same relative order of potency as β_1-adrenoceptors (Table 1) but is distinguished in this regard by its high responsiveness to BRL 37344. A similar conclusion may be drawn from the results of Coleman *et al.* (1987) in the guinea-pig stomach fundus.

In view of the selectivity and potency of BRL 37344 as a lipolytic agent (Arch *et al.*, 1984; Wilson *et al.*, 1984) and reports that β-adrenoceptors on fat cells are atypical (Arch *et al.*, 1984; Bojanic *et al.*, 1984; Harms *et al.*, 1977; Wilson *et al.*, 1984) the question arises as to whether the putative adrenoceptor in the guinea-pig ileum and the atypical β-adrenoceptor on adipocytes are the same. Evidence in support of this notion is provided by the similar rank order and relative potency of agonists at the putative adrenoceptor in the ileum and at the atypical β-adrenoceptor in rat adiopocytes. Thus, Arch *et al.* (1984) reported the following order and relative potencies for lipolysis: BRL 37344 (1) > isoprenaline (0.2) > fenoterol (0.008), and Wilson *et al.* (1984), using BRL 35135, which is hydrolysed to the active free acid, BRL 37344, reported: BRL 35135 (1) = isoprenaline (1) > fenoterol (0.03). Likewise, Bojanic *et al.* (1985) found noradrenaline to be about 10 times more potent than fenoterol at stimulating lipolysis in rat adipocytes. On the other hand, there is one important piece of evidence which argues against the notion that the adipocyte and ileal adrenoceptor are the same. This comes from the data obtained with propranolol. The atypical β-adrenoceptor on fat cells is sensitive to blockade by propranolol, but abnormally low pA_2 values (6.2 to 6.6) have been reported (Wilson *et al.*, 1984). In contrast, the putative adrenoceptor in guinea-pig ileum is totally resistant to propranolol at 5 μM (present study), and at 10 μM (Bond *et al.*, 1986a). That is, the putative adrenoceptor is resistant to propranolol at concentrations that are at least 8 to 16 times its equilibrium dissociation constant for the adipocyte receptor. A 1 log unit difference between equilibrium dissociation constants for an antagonist may be taken as strong evidence for distinct sites (Furchgott, 1972; Eglen & Whiting, 1987). However, it would be premature to discriminate the two receptors solely upon the basis of propranolol. Further quantitative and comparative studies with other β-adrenoceptor

antagonists are needed (e.g.: the determination of the pA_2 value for nadolol at the adrenoceptor mediating lipolysis in rat adipocytes).

There are several other examples in the literature of β-adrenoceptor-like responses to phenylethylamine based agonists which are resistant to α- and β-adrenoceptor blockade (Morris *et al.*, 1981; Drew & Hilditch, 1984; Broadley *et al.*, 1985; Bentley & Starr, 1986; Dettmar *et al.*, 1986; Croci *et al.*, 1988). The study by Croci *et al.* (1988) is especially noteworthy in that these authors appear to have identified a putative β-adrenoceptor subtype in rat colon which is stimulated selectively by a series of novel agonists (phenylethanolaminotetralines). But, as with the putative adrenoceptor and the adrenoceptor mediating lipolysis, it may be premature to claim novel sites. For instance, the putative adrenoceptor may represent immature or aging β-adrenoceptors in various stages of their membrane life cycle (Mahan *et al.*, 1987; Maisel *et al.*, 1987) or receptor domain may serve to confer unique properties upon interacting drugs (Ariëns *et al.*, 1979; Kenakin, 1984). It is important to stress that both the putative adrenoceptor identified in the guinea-pig ileum and the atypical β-adrenoceptor on adipocytes exhibit strong similarities to the β_1-adrenoceptor. Endogenously occurring agonists (noradrenaline and adrenaline) and agonists with only modest structural differences (isoprenaline) are read in the same relative order (Table 1; Lands *et al.*, 1967b). Accessory binding sites located within the domain of the receptor may come into play with more complex synthetic agonists and antagonists (compounds of large molecular size) so as to influence potency and affinity respectively, thereby 'creating' an apparently novel effector site. Until the putative adrenoceptor in guinea-pig ileum has been shown to exhibit a different affinity for noradrenaline and adrenaline from that at the β_1-adrenoceptor it will be very difficult to make an unequivocal claim for a new receptor despite the clear distinctions seen with propranolol and nadolol. This notion would be in full accord with Stephenson's definition of the term receptor: 'that small spatial arrangement of atoms to which a substance endogenous to the organism attaches itself as an essential step in modifying cellular functions' (Stephenson, 1975).

In a different vein is the notion that the receptor site defined in the present study may subserve a primary function for a neurotransmitter or hormone different from noradrenaline and adrenaline. In this regard, inhibitory receptors for 5-hydroxytryptamine in the guinea-pig ileum (Feniuk *et al.*, 1983; Kalkman *et al.*, 1986) can be ruled out as responses to isoprenaline were not antagonized by methysergide (3 μM, unpublished observations). However, the gut contains at least 18 putative neurotransmitters or co-transmitters (Burnstock, 1985) and is subjected to the actions of several circulating hormones, with parathyroid hormone producing similar responses to β-adrenoceptor agonism (Pang *et al.*, 1986).

In conclusion, the present study has characterized a putative adrenoceptor in guinea-pig ileum which can be distinguished pharmacologically from currently defined α- and β-adrenoceptors. This putative receptor responds to phenylethylamine based agonists in the following order of potency: BRL 37344 (20) > (−)-isoprenaline (8) > noradrenaline (1) > adrenaline (0.5) > fenoterol (0.35) > (+)-isoprenaline (0.27) and is inhibited by nadolol with a mean pA_2 value of 4.71 (slopes of Schild regressions constrained to 1). Because of the complexities involved it would be unwise to name this site using specific nomenclature (e.g.: β_3-adrenoceptor). Instead, we intend to refer to the putative adrenoceptor as an 'atypical' β-adrenoceptor in guinea-pig ileum.

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