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Characterization of atypical β-adrenoceptors in the guinea pig duodenum

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Abstract

The atypical β-adrenoceptors mediating relaxation in the guinea pig duodenum were studied using catecholamines (isoprenaline, noradrenaline and adrenaline), a selective β_3-adrenoceptor agonist BRL37344 ((*R**,*R**)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium salt) and a non-conventional partial β_3-adrenoceptor agonist CGP12177A ((−)-4-(3-*t*-butylamino-2-hydroxypropoxy)benzimidazol-2-one)). Catecholamines and β_3-adrenoceptor agonists induced concentration-dependent relaxation in this preparation. Propranolol (1 μM) produced only small rightward shifts in the concentration–response curves of these agonists. In the presence of propranolol (1 μM), however, a non-selective β_1-, β_2- and β_3-adrenoceptor antagonist bupranolol caused a concentration-dependent rightward shift of the concentration–response curves for catecholamines and β_3-adrenoceptor agonists. Schild plot analyses of the effects of bupranolol against these agonists gave pA_2 values of 6.02 (isoprenaline), 5.98 (noradrenaline), 5.93 (adrenaline), 6.51 (BRL37344) and 5.70 (CGP12177A), respectively, and all Schild slopes were not significantly different from unity. These results suggest that atypical β-adrenoceptors are present in the guinea pig duodenum and involved in mediating the functional relaxant response.

Keywords: β-Adrenoceptor, atypical; β_3-Adrenoceptor; Duodenum, guinea pig; BRL37344; CGP12177A; Bupranolol

1. Introduction

β-Adrenoceptors were first subdivided by Lands et al. (1967a,b) into β_1- and β_2-adrenoceptor subtypes based on the relative potencies of catecholamine agonists. Subsequently, both pharmacological studies and molecular cloning techniques have revealed the existence of atypical β-adrenoceptors, or β_3-adrenoceptors, different from classical β_1- and/or β_2-adrenoceptors (for review see Arch and Kaumann, 1993). In the gastrointestinal tissue atypical β-adrenoceptors mediate relaxation of smooth muscle in response to catecholamines and selective β_3-adrenoceptor agonists (for review see Manara et al., 1995).

We have reported that β_2- and β_3-adrenoceptors are involved in the β-adrenoceptor-mediated relaxation of the guinea pig taenia caecum (Koike et al., 1994, 1995a,b). In addition, our previous studies also showed that the relaxant responses to BRL37344 [(*R**,*R**)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid] (CGP1277A), [(−)-4-(3-*t*-butylamino-2-hydroxypropoxy)-benzimidazol-2-one] and noradrenaline in the guinea pig taenia caecum were mediated by β_3-adrenoceptors and were competitively antagonized by a non-selective β-adrenoceptor antagonist, bupranolol (pA_2 5.79, 5.61 and 5.53, respectively, Koike et al., 1995b, 1997), although at a concentration much higher than that necessary for the blockade of β_1- or β_2-adrenoceptors (Kaumann, 1989). However, there is no evidence for the presence of atypical β-adrenoceptors in the guinea pig duodenum. Moreover, several pA_2 values for bupranolol have been reported: the value against CGP12177A is 5.7 in rat brown adipose tissue (Malinowska and Schlicker, 1997) and is 6.70 in rat fat cell (Galitzky et al., 1997). It remains possible that there is a species difference or a tissue difference in the presence of atypical β-adrenoceptors.

The purpose of the present study was to characterize the atypical β-adrenoceptors involved in relaxant responses in the guinea pig duodenum in functional experiments with catecholamines (isoprenaline, noradrenaline and adrenaline), β_3-adrenoceptor agonists (BRL37344 and CGP12177A) and a β_3-adrenoceptor antagonist bupranolol, and to obtain further evidence to clarify whether there is a tissue difference in atypical β-adrenoceptors in the guinea pig gastrointestinal tissue systems.

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2. Methods

2.1. Mechanical responses

Male guinea pigs weighing 300–500 g were killed by cervical dislocation and the entire duodenum was rapidly isolated and placed in oxygenated (a mixture of 95% O_2 and 5% CO_2) Ringer–Locke solution of the following composition (in mM): NaCl, 154; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 2.1; $NaHCO_3$, 5.9 and glucose, 2.8. The luminal contents were removed immediately and the connective tissue was dissected away. The outer layer of duodenum containing longitudinal smooth muscle was carefully removed with a cotton swab. Each strip (10 mm in length) was set up in a 20-ml organ bath at 32°C containing Ringer–Locke solution bubbled with a mixture of 95% O_2 and 5% CO_2. The mechanical responses of strips were recorded isometrically under a load of 0.5 g. Desmethylimipramine (1 μM), normetanephrine (10 μM) and phentolamine (10 μM) were present throughout the experiments to inhibit neuronal uptake, extraneuronal uptake and α-adrenoceptors, respectively. Strips were incubated for at least 30 min before any experimental procedures were begun. In some experiments, we used guinea pig taenia caecum. This method was described by Koike et al. (1997).

After two histamine concentration–response curves had been obtained, the relaxant responses of agonists were determined by measuring the inhibition of the histamine (10 μM)-induced submaximal contraction by addition of the agonists. β-Adrenoceptor agonists were added cumulatively until a maximal relaxant response was observed, and the relaxation induced by these drugs was expressed as a percentage of the maximal relaxation produced by isoprenaline (3 μM), the reference drug.

To assess the antagonist effect, one of the antagonists was added to the bath 30 min before the addition of the agonist. The concentration–response curves for the agonist were then obtained in the presence of an antagonist. The time interval between two consecutive curves was usually set at 30 min. In our previous experiments, after the control concentration–response curves were determined, four or five successive cumulative concentration–response curves for catecholamines were determined. The curves were nearly superimposable and changes in sensitivity (sensitization or desensitization) were slight (data not shown). Seven or more concentration–response curves could be made in succession. However, CGP12177A caused desensitization (Kaumann, 1996) and BRL37344 caused tachyphylaxis (Coleman et al., 1987). Therefore, antagonist effects on the relaxant responses of these agonists were assessed by using paired tissues, i.e., one tissue where the concentration–response curve for the agonist (CGP12177A or BRL37344) was measured in the absence of antagonist and the other tissue where the same procedure was carried out in the presence of the antagonists. The procedure helped to assess the contribution of other factors such as desensitization and tachyphylaxis. Agonistic potency is expressed as the pD_2 value (Van Rossum, 1963). The intrinsic activity of each agonist was calculated as the ratio of the maximal relaxation induced by each agonist to the maximal relaxation induced by the full agonist isoprenaline (3 μM). The competitive antagonistic potency is expressed as the pA_2 value. It was calculated according to the method of Tallarida et al. (1979), which was originally described by Arunlakshana and Schild (1959).

2.2. Data analysis

All results are presented as means ± S.E. of 6–8 experiments. Statistical analyses were performed with Newman–Keul's test when appropriate. A P value of less than 0.05 was considered significant.

2.3. Drugs

The following drugs were used in the current study: isoprenaline hydrochloride, noradrenaline bitartrate, adrenaline bitartrate, propranolol hydrochloride, histamine dihydrochloride, desmethylimipramine hydrochloride, normetanephrine hydrochloride (Sigma, St. Louis, MO, USA); CGP12177A (Research Biochemicals, Natick, MA, USA); phentolamine mesylate (Ciba, Basal, Switzerland); bupranolol hydrochloride was kind gift from Kaken Seiyaku (Tokyo, Japan); and BRL37344 (Nacalaitesque, Kyoto, Japan). All the drugs were dissolved in distilled water. The other chemicals used were of analytical grade.

3. Results

3.1. Responses to catecholamines

In the absence of propranolol (1 μM), the three catecholamines produced concentration-dependent relaxations of the guinea pig duodenum (Fig. 1a). The pD_2 values and intrinsic activities are shown Table 1. Noradrenaline and adrenaline were partial agonists since their intrinsic activities (0.83 ± 0.03 and 0.79 ± 0.03, respectively) were significantly lower than 1. The relative rank order of potency was isoprenaline > noradrenaline > adrenaline.

In the presence of propranolol (1 μM) only small rightward shifts of the concentration–response curves were observed (isoprenaline, 15-fold shift, noradrenaline 3-fold shift, adrenaline, 3-fold shift), giving an order of potency of isoprenaline > noradrenaline > adrenaline (Fig. 1b, Table 1). Propranolol (1 μM) had no significant effect on the maximum relaxant responses to catecholamines (Fig. 1b, Table 1).

3.2. Responses to β_3-adrenoceptor agonists

In the absence of propranolol (1 μM), a concentration-dependent relaxation in the guinea pig duodenum was

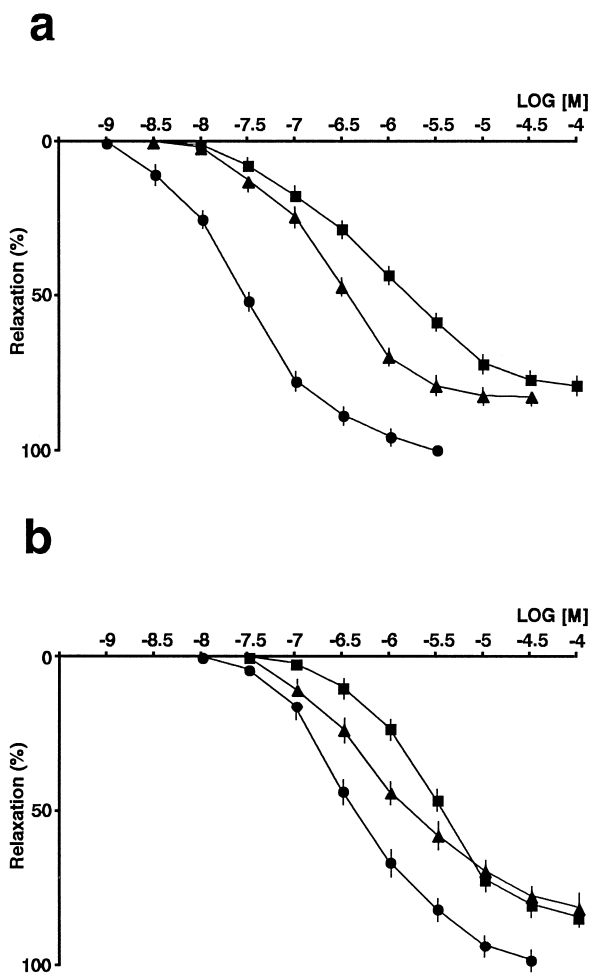


Fig. 1. Concentration–response curves for isoprenaline, noradrenaline and adrenaline (a) in the absence of propranolol and (b) in the presence of propranolol (1 μM). Isoprenaline (●); noradrenaline (▲); adrenaline (■). Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation induced by isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E. of 6–8 experiments.

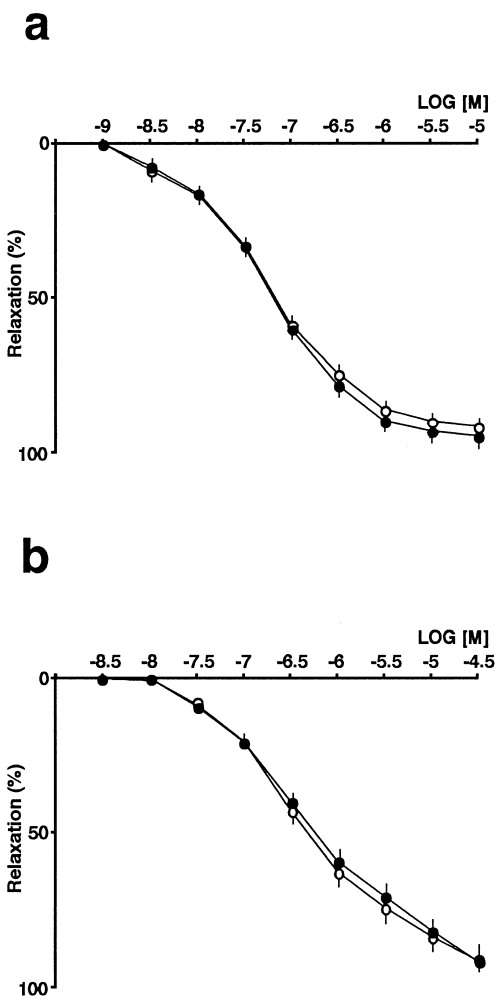


Fig. 2. Effect of propranolol (1 μM) on concentration–response curves for BRL37344 and CGP12177A. (a) BRL37344 only (○); BRL37344 + propranolol (1 μM) (●). (b) CGP12177A only (○); CGP12177A + propranolol (1 μM) (●). Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation induced by isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E. of 6–8 experiments.

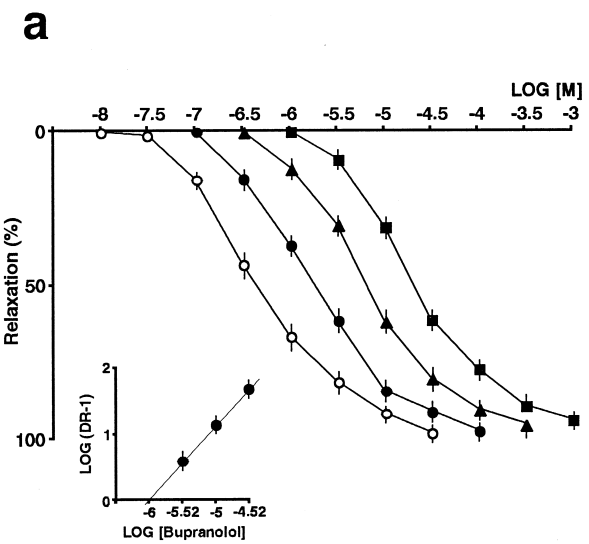
produced by the two β_3-adrenoceptor agonists (Fig. 2a,b). BRL37344 was similar in potency to isoprenaline, and CGP12177A was somewhat less potent than isoprenaline (Table 1). The intrinsic activities of BRL37344 and CGP12177A were 0.89 ± 0.02 and 0.92 ± 0.01, respectively, indicating that these agonists acted as partial agonists in this preparation. The concentration–response curves for BRL37344 and CGP12177A were unaffected by propranolol (1 μM, Fig. 2a,b).

Table 1
The pD_2 values and intrinsic activities of catecholamines and β_3-adrenoceptor agonists on the guinea pig duodenum in the absence or presence of propranolol (1 μM)
Values are means ± S.E. from 6–8 separate experiments. IA: intrinsic activity (maximum relaxation induced by each agonist in the absence or presence of propranolol (1 μM) with respect to the maximum relaxation induced by the full agonist isoprenaline (3 μM) in the absence of propranolol). pD_2 value = $-\log EC_{50}$ (EC_{50}: concentration of agonist inducing half-maximum relaxation).

Agonist	Absence of propranolol		Presence of propranolol	
	pD_2 value	IA	pD_2 value	IA
Isoprenaline	7.54 ± 0.03	1	6.38 ± 0.05	0.98 ± 0.04
Noradrenaline	6.63 ± 0.05	0.83 ± 0.03	6.12 ± 0.04	0.81 ± 0.04
Adrenaline	6.16 ± 0.06	0.79 ± 0.03	5.65 ± 0.03	0.84 ± 0.02
BRL37344	7.29 ± 0.02	0.89 ± 0.02	7.24 ± 0.04	0.94 ± 0.01
CGP12177A	6.43 ± 0.02	0.92 ± 0.01	6.43 ± 0.03	0.91 ± 0.04

3.3. Effect of β_3-adrenoceptor antagonist bupranolol

In the presence of propranolol (1 μM), the β_3-adrenoceptor antagonist bupranolol (3–30 μM) competitively antagonized relaxant responses to catecholamines and β_3-adrenoceptor agonists (Fig. 3a–e). The Arunlakshana–Schild plot of the data revealed the pA_2 values for bupranolol against catecholamines and β_3-adrenoceptor agonists to be 6.02 ± 0.02 (isoprenaline, slope; 1.08 ± 0.04),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Relaxation (%)
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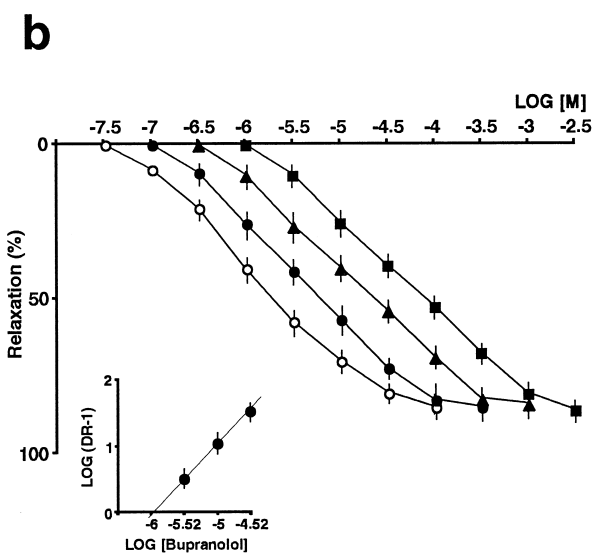
50
100
LOG (DR-1)
2
1
0
-6 -5.52 -5 -4.52
LOG [Bupranolol]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Relaxation (%)
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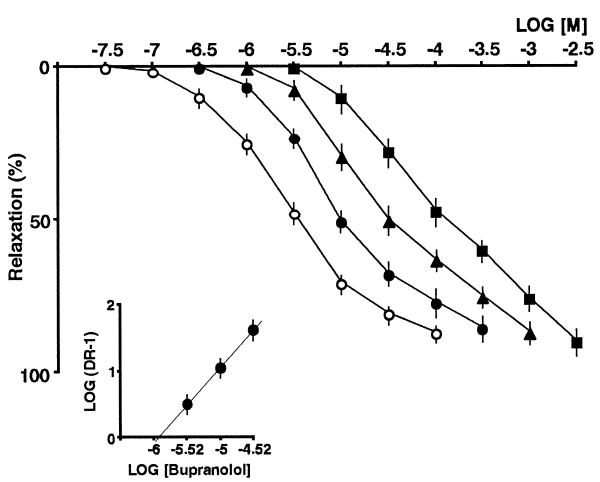
50
100
LOG (DR-1)
2
1
0
-6 -5.52 -5 -4.52
LOG [Bupranolol]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Relaxation (%)
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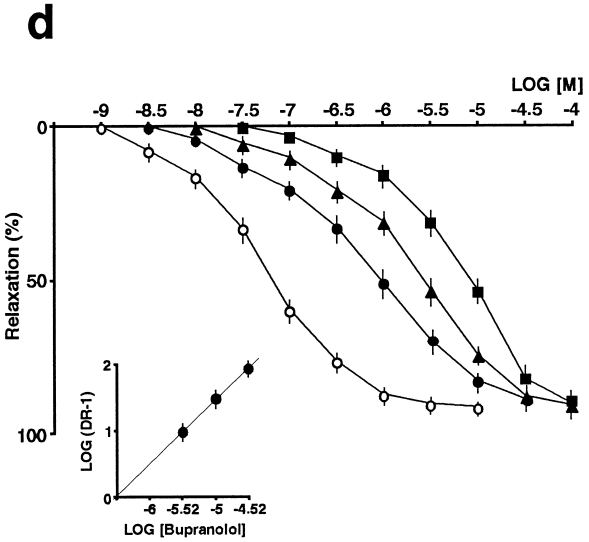
50
100
LOG (DR-1)
2
1
0
-6 -5.52 -5 -4.52
LOG [Bupranolol]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Relaxation (%)
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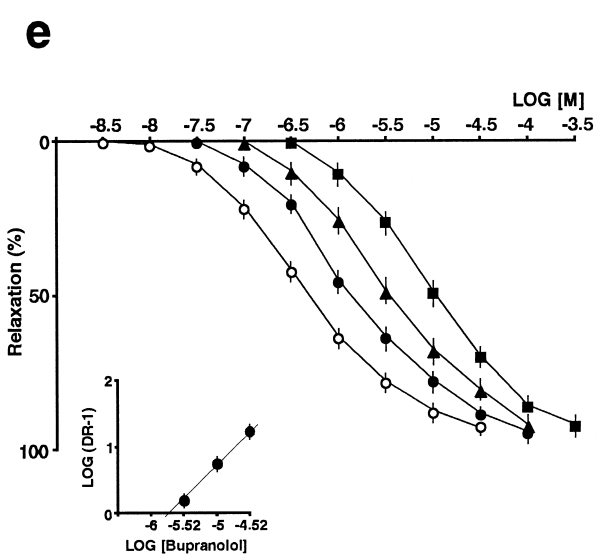
50
100
LOG (DR-1)
2
1
0
-6 -5.52 -5 -4.52
LOG [Bupranolol]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Relaxation (%)
0
50
100
LOG (DR-1)
2
1
0
-6 -5.52 -5 -4.52
LOG [Bupranolol]

5.98 ± 0.06 (noradrenaline, 1.00 ± 0.05), 5.93 ± 0.08 (adrenaline, 1.09 ± 0.06), 6.51 ± 0.02 (BRL37344, 0.97 ± 0.02) and 5.70 ± 0.06 (CGP12177A, 1.09 ± 0.05). The slope of each regression line was not significantly different from unity. In the guinea pig taenia caecum the pA_2 value for bupranolol against BRL37344 was 5.74 ± 0.03, the slope (1.01 ± 0.03) not being significantly different from unity.

4. Discussion

In the present experiment, we aimed to obtain pharmacological evidence of the presence of atypical β-adrenoceptors in the guinea pig duodenum by using catecholamines and β_3-adrenoceptor agonists. Comparing the pA_2 values for bupranolol against these agonists, we attempted to determine whether atypical β-adrenoceptors in the guinea pig duodenum are different from those previously characterized in the guinea pig taenia caecum (Koike et al., 1995b, 1997).

In the absence of propranolol, catecholamines induced concentration-dependent relaxation with an order of potency corresponding to their rank order of potency in tissues in which β_3-adrenoceptor responses are observed (MacDonald et al., 1994). The responses were mainly β-adrenoceptor in nature since isoprenaline was more potent than noradrenaline and adrenaline. Furthermore, noradrenaline was more potent than adrenaline, indicating an atypical β-adrenoceptor-mediated response. In the presence of propranolol (1 μM), only small rightward shifts of the concentration–response curves for the catecholamines were observed. However, the order of potency of catecholamines did not change. These results suggested that atypical β-adrenoceptors were quantitatively more abundant than classical β_1- or/and β_2-adrenoceptors and therefore in dominant control of responses to the three agonists, especially endogenous mediators noradrenaline and adrenaline.

Since a selective β_3-adrenoceptor agonist BRL37344 and a non-conventional partial β_3-adrenoceptor agonist CGP12177A (a potent β_1- and β_2-adrenoceptor antagonist with a partial β_3-adrenoceptor agonist action) have been used as reference atypical β-adrenoceptor agonists in guinea pig gastrointestinal tissue (Koike et al., 1995b, 1997), we determined whether the relaxant responses of these agonists in the guinea pig duodenum were mediated via atypical β-adrenoceptors. The relaxant effects of BRL37344 and CGP12177A were resistant to propranolol (1 μM), indicating the presence of propranolol-insensitive responses. Subsequently, BRL37344 was shown to be approximately 7-fold more potent than isoprenaline and CGP12177 was as potent as isoprenaline in eliciting these propranolol-resistant responses, suggesting that they were mediated by atypical β-adrenoceptors. These results were in agreement with the rank order of potency, BRL37344 > isoprenaline > noradrenaline > adrenaline, reported by Bond and Clarke (1988) for the guinea pig ileum.

To define an atypical β-adrenoceptor Arch and Kaumann (1993) established three criteria: (i) the receptor should be selectively stimulated by β_3-adrenoceptor-selective agonists (e.g., BRL37344), (ii) the receptor should be stimulated by non-conventional partial agonists (e.g., CGP12177A) and (iii) the receptor should be resistant to blockade by antagonists possessing only high affinity for β_1- and β_2-adrenoceptors. Our present results suggest that all three criteria were fulfilled for the catecholamine- and β_3-adrenoceptor agonist-induced relaxation of the guinea pig duodenum.

In this study, the non-selective β-adrenoceptor antagonist bupranolol was used to block atypical β-adrenoceptors (Arch and Kaumann, 1993). The selectivity of bupranolol at β-adrenoceptor subtypes is $\beta_2 > \beta_1 >$ atypical β/β_3 (Kaumann and Molenaar, 1996). To detect atypical β-adrenoceptors using bupranolol, therefore, we set up functional competition experiments with propranolol (1 μM) to block classical β_1- and β_2-adrenoceptors. These conditions were used in some previous studies (Koike et al., 1994). The relaxant responses to catecholamines and β_3-adrenoceptors agonists were competitively antagonized by bupranolol. All Schild plots were linear with slopes not different from unity, indicating a competitive form of antagonism at atypical β-adrenoceptors in the guinea pig duodenum. Calculated pA_2 values for bupranolol against isoprenaline, noradrenaline, adrenaline, BRL37344 and CGP12177A were 6.02, 5.98, 5.93, 6.51 and 5.70, respectively. Each pA_2 value for bupranolol against noradrenaline and CGP12177A obtained in the present study was not significantly different from its value in the guinea pig taenia caecum ($P < 0.05$, Koike et al., 1994, 1995b); however, the pA_2 value against BRL37344 was significantly different from the pA_2 value in the guinea pig taenia caecum ($P < 0.05$). These results suggest that there is a difference between atypical β-adrenoceptors in the guinea pig duodenum and the guinea pig taenia caecum. It is also possible that there are two subtypes of atypical β-adrenoceptors in the guinea pig duodenum, and that bupranolol recognizes the two receptor subtypes, one of which is stimulated by BRL37344 and the other by CGP12177A.

Recently, evidence has accumulated for the existence of a 'putative' β_4-adrenoceptor which is pharmacologically

Fig. 3. Effect of bupranolol on concentration–response curves for isoprenaline (a), noradrenaline (b), adrenaline (c), BRL37344 (d) and CGP12177A (e) in the presence of propranolol (1 μM). Control, no bupranolol (○); bupranolol 3 μM (●); bupranolol 10 μM (▲); bupranolol 30 μM (■). Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation induced by isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E. of 6–8 experiments. The insets show the corresponding Schild plots.

distinct from the β_1-, β_2- and β_3-adrenoceptor subtypes in rat atrium (Sarsero et al., 1998) and human fat cells (Galitzky et al., 1997). The 'putative' β_4-adrenoceptor is selectively stimulated by CGP12177A whereas other β_3-adrenoceptor agonists (BRL37344, CL316243, ZD2079 and SR58611A) do not have stimulant effects (Kaumann and Molenaar, 1996). This effect of CGP12177A is blocked by bupranolol and is resistant to selective blockade with the new β_3-adrenoceptor antagonist SR59230A (Kaumann and Molenaar, 1996; Galitzky et al., 1997). In the guinea pig duodenum BRL37344 stimulated atypical β-adrenoceptor subtypes antagonized by bupranolol, indicating that the relaxant responses of BRL37344 are mediated through atypical β-adrenoceptor subtypes pharmacologically different from the 'putative' β_4-adrenoceptor.

In conclusion, the present study provides functional evidence for the existence of atypical β-adrenoceptors in the guinea pig duodenum. The resistance of catecholamine- and β_3-adrenoceptor agonist-induced relaxant responses to propranolol, the relatively high potency of β_3-adrenoceptor agonists compared to catecholamines, and the competitive antagonism of relaxant responses to catecholamines and β_3-adrenoceptor agonists by bupranolol suggest that the β-adrenoceptors in this tissue are mainly atypical β-adrenoceptors. It is possible that BRL37344 induces relaxation in the guinea pig duodenum through the stimulation of atypical β-adrenoceptors pharmacologically distinct from the atypical β-adrenoceptors in the guinea pig taenia caecum.

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