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European Journal of Pharmacology 416 (2001) 153–163

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Functional properties of atypical β-adrenoceptors on the guinea pig duodenum

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Received 23 November 2000; received in revised form 20 February 2001; accepted 26 February 2001

Abstract

In this study, we attempted to further characterize atypical β-adrenoceptors on the guinea pig duodenum. (−)-Enantiomers of isoprenaline and noradrenaline were more potent than its (+)-enantiomers. The isomeric activity ratios ((+)/(−)) were less than those obtained in the guinea pig atria and trachea. The concentration–response curves to catecholamines ((−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline), to the selective β_3-adrenoceptor agonist, BRL37344 ((*R**, *R**)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium), and to the non-conventional partial β_3-adrenoceptor agonist, (±)-CGP12177A ((±)-[4-[3-[(1,1-dimethylethyl)-amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-benzimidazol-2-one] hydrochloride), were resistant to blockade by (±)-pindobind, the β-adrenoceptor alkylating agent. (−)-Noradrenaline and (−)-adrenaline were more potent than dopamine and (−)-phenylephrine, respectively. Selective β_2-adrenoceptor agonists possess agonistic activities at atypical β-adrenoceptors. (±)-Propranolol and (±)-bupranolol had no agonistic effect, whereas (±)-alprenolol, (±)-pindolol, (±)-nadolol, (±)-CGP12177A and (±)-carteolol exhibited agonistic activities at atypical β-adrenoceptors. These results suggest that pharmacological properties of atypical β-adrenoceptors differ from those of conventional β_1- and β_2-adrenoceptors on the guinea pig. © 2001 Published by Elsevier Science B.V.

Keywords: β-adrenoceptor, atypical; β_3-Adrenoceptor; Duodenum, guinea pig; Stereoselectivity; β-Adrenoceptor alkylating; Structure–activity relationship

1. Introduction

Atypical β-adrenoceptors have been identified by functional, molecular cloning and receptor binding studies in several gastrointestinal tract (for review see Manara et al., 1995) and adipose tissues (for review see Arch and Kaumann, 1993). Since this β-adrenoceptor subtype possesses an unusually low affinity for the typical β-adrenoceptor antagonists including propranolol, these receptors have been termed atypical β-adrenoceptors.

Recently, we have suggested that atypical β-adrenoceptors were involved in mediating the relaxant response of the guinea pig duodenum (Horinouchi and Koike, 1999a). We also demonstrated that the relaxant responses to catecholamines ((−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline), to a selective β_3-adrenoceptor agonist, BRL37344 ((*R**, *R**)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium salt), and to the non-conventional partial β_3-adrenoceptor agonist, (±)-CGP12177A ((±)-[4-[3-[(1,1-dimethylethyl)-amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-benzimidazol-2-one] hydrochloride), were resistant to blockade by the non-selective β_1- and β_2-adrenoceptor antagonist, (±)-propranolol (1 μM) (Horinouchi and Koike, 1999a). However, the relaxant responses to the five agonists were competitively antagonized by a non-selective β_1-, β_2- and β_3-adrenoceptor antagonist, (±)-bupranolol (Horinouchi and Koike, 1999a), although at concentrations greatly exceeding those required for β_1- or β_2-adrenoceptor blockade (Kaumann, 1989).

The atypical β-adrenoceptor-mediated responses are characterized by low stereoselectivity ratios of antagonist enantiomers (Zaagsma and Nahorski, 1990; Emorine et al., 1994), while both β_1- and β_2-adrenoceptors exhibit high stereoselectivity (for review see Ruffolo, 1991). However,

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0014-2999/01/$ - see front matter © 2001 Published by Elsevier Science B.V.
PII: S0014-2999(01)00877-9

the isomeric activity ratio of trimetoquinol (the selective β_2-adrenoceptor agonist) isomers in atypical β-adrenoceptors of rat esophageal smooth muscle was greater than that obtained in β_1-adrenoceptors of rat atria and β_2-adrenoc-

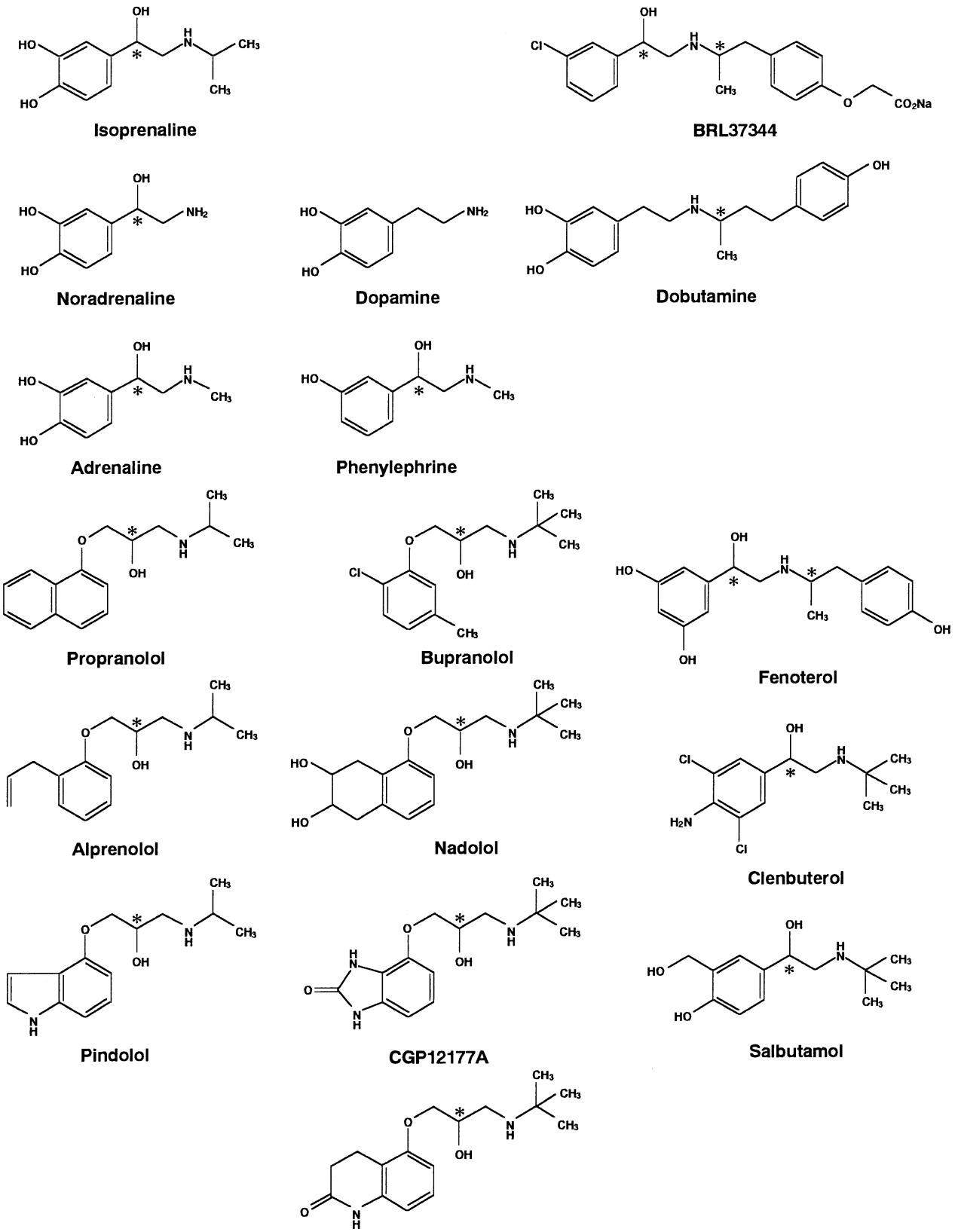

Fig. 1. Chemical structures formula of various drugs used in the present study. The presence of an asymmetric carbon atom is denoted by an asterisk (*).

eptors of rat trachea (Lezama et al., 1996). Trimetoquinol is a prototype of the tetrahydroisoquinoline class of compounds that is structurally distinct from catecholamines (Lezama et al., 1996). It lacks the β-hydroxyl group of catecholamines and the asymmetric carbon atom is contained within a semirigid tetrahydroisoquinoline ring (Lezama et al., 1996). It is possible that the isomeric activity ratio of stereoisomers at atypical β-adrenoceptors depend on the position of asymmetric carbon atom.

(±)-Propranolol, (±)-alprenolol, (±)-pindolol, (±)-bupranolol, (±)-nadolol, (±)-CGP12177A and (±)-carteolol belong to aryloxypropanolamine class (see Fig. 1 for chemical structure). The chemical structure of the above drugs closely resembles each other. However, the drugs exhibited a variety of functional responses at atypical β/β_3-adrenoceptors. (±)-Bupranolol possesses antagonistic activities at atypical β-adrenoceptors (Kaumann, 1989; Horinouchi and Koike, 1999a,b), whereas (±)-CGP12177A behaves as an agonist at atypical β-adrenoceptors in the guinea pig duodenum and gastric fundus (Horinouchi and Koike, 1999a,b). Blin et al. (1993) showed that (±)-pindolol possessed agonistic properties with an intrinsic activity of 0.55 at β_3-adrenoceptors in Chinese hamster ovary cells expressing the human β_3-adrenoceptors. However, Hoey et al. (1996a) had reported that (±)-pindolol acts as an antagonist at β_3-adrenoceptors in the rat ileum. Furthermore, (±)-carteolol possesses both agonistic and antagonistic activities at atypical β-adrenoceptors on the guinea pig duodenum (Horinouchi and Koike, 2000) and at β_3-adrenoceptors on brown fat cells from mouse, rat and hamster (Zhao et al., 1998). Recently, the aryloxypropanolamines SB-226552 and its analogues, which resemble (±)-CGP12177A structurally, have been developed as the selective β_3-adrenoceptor agonist for Chinese hamster ovary cells expressing the human β_3-adrenoceptors (Sennitt et al., 1998). Thus, aryloxypropanolamine compounds show various activities at atypical β/β_3-adrenoceptors. We therefore considered that the aryloxypropanolamine compounds possesses the agonistic activity at atypical β-adrenoceptors.

The objective of the present study is to further characterize atypical β-adrenoceptors in the guinea pig duodenum. Since recent studies suggested that atypical β-adrenoceptors could become a target for the development of new drugs in the treatment of obesity (Arch et al., 1984), gastrointestinal tract disorders (Anthony, 1996; Bahl et al., 1996) and non-insulin-dependent diabetes (Yamamoto et al., 1997), the determination of stereochemical specificity for atypical β-adrenoceptors would be important to avert their side-effects of potential clinical relevance (Giudice et al., 1989). Therefore, we have used enantiomers of catecholamines (isoprenaline, noradrenaline and adrenaline) and aryloxypropanolamines (alprenolol and pindolol) in order to examine the stereochemical requirements of atypical β-adrenoceptors in the guinea pig duodenum. In addition, we have carried out the structure–activity relationship studies to clarify the chemical structure requirements of atypical β-adrenoceptors. Selective β_2-adrenoceptor agonists ((±)-fenoterol, (±)-clenbuterol and (±)-salbutamol), which are similar to BRL37344, have been used to clarify whether the three drugs act with agonistic activity at atypical β-adrenoceptors. Moreover, in order to assess the agonistic effects of aryloxypropanolamines at atypical β-adrenoceptors, we have carried out functional experiments, which were established by Horinouchi and Koike (1999a) for atypical β-adrenoceptors in the guinea pig duodenum.

2. Materials and methods

2.1. Animals and tissue preparation

Male Hartley guinea pigs weighing 300–500 g (Saitama Experimental Animals, Saitama, Japan) were used in accordance with the Guide for the Care and Use of Laboratory Animals of Toho University School of Pharmaceutical Sciences, and the protocol of the present study was approved by the Institutional Animal Care and Use Committee. Guinea pigs were housed under laboratory standard conditions on a 12-h light/dark cycle (lights on 8:00 AM; lights off 8:00 PM) in temperature- (20–22°C) and relative air humidity- (50 ± 5%) controlled room. Food and water were available ad libitum.

Guinea pigs were sacrificed by cervical dislocation and the duodenum was isolated. 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. Strips (10 mm in length) were mounted vertically under an initial tension of 0.5 g in a 20-ml organ bath containing Ringer–Locke solution (NaCl, 154; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 2.1; $NaHCO_3$, 5.9 and glucose, 2.8 mM), maintained at 32°C and bubbled continuously with a mixture of 95% O_2 and 5% CO_2 (pH 7.4). Imipramine (1 μM, a neuronal uptake inhibitor), normetanephrine (10 μM, an extraneuronal uptake inhibitor), phentolamine (10 μM, an α-adrenoceptor antagonist) and ascorbic acid (10 μM, to prevent oxidation of catecholamines) were present in the medium throughout all experiments.

2.2. Experimental protocols

After the preparations were allowed to equilibrate for 30 min in the absence of β-adrenoceptor antagonist, the preparations were contracted with histamine (10 μM), which induced a contraction equal to 70–80% of the maximal histamine-induced contraction. The β-adrenoceptor-mediated relaxations caused by test drugs were determined by measuring the inhibition of the histamine-induced contraction. Firstly, concentration–response curves

for (−)-isoprenaline (up to 3 μM) were generated as controls (100%). Histamine (10 μM) was added to the bath 30 min after washing out the drug, then test drugs 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 the reference drug, (−)-isoprenaline (3 μM), in the absence of β-adrenoceptor antagonist.

In order to assess the atypical β-adrenoceptor-mediated relaxations, the non-selective β_1- and β_2-adrenoceptor antagonist, (±)-propranolol (1 μM), were added to the bath 60 min before the cumulative addition of the test drug. (±)-Propranolol (1 μM) itself did not induce the inhibition of the histamine-induced contraction (data not shown). In preliminary experiments, the tissue sensitivity and the maximum response to several test drugs except catecholamines (stereoisomers of isoprenaline, noradrenaline and adrenaline) decreased when two consecutive concentration–response curve for these drugs were performed with the same segment (data not shown); therefore, a single cumulative concentration–response curve to each test drug was made for each strip.

2.3. β-Adrenoceptor alkylating experiments

The method of Molenaar et al. (1988) was used. Briefly, preparations were incubated with (±)-pindobind (10 μM), the β-adrenoceptor alkylating drug, for 90 min, then, preparations were washed with Ringer–Locke solution every 5 min for 30 min. After the preparations were allowed to equilibrate for 30 min in the presence of (±)-propranolol (1 μM), the preparations were contracted with histamine, which induced a contraction equal to 70–80% of the maximal histamine-induced contraction. Then concentration–response curves to test drugs were established.

2.4. Data analysis

The results are expressed as means ± S.E.M. of the number (n) of experiments. Agonistic potency was ex-

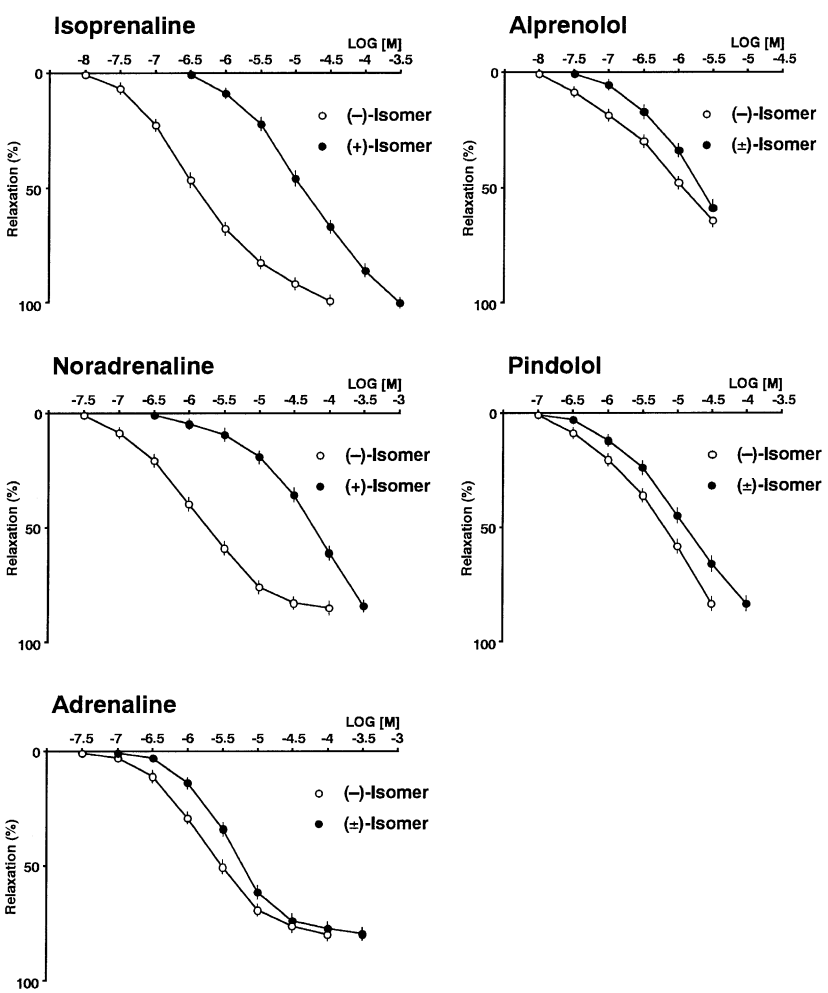

Fig. 2. Concentration–response curves for stereoisomers of catecholamines (isoprenaline, noradrenaline and adrenaline) and aryloxypropanolamines (alprenolol and pindolol) in the presence of (±)-propranolol (1 μM). Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation (in the absence of (±)-propranolol (1 μM)) induced by (−)-isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E.M. of n experiments. The n values and the results are shown in Table 1.

pressed as the pD_2 value (Van Rossum, 1963). The intrinsic activity of each drug was calculated as the ratio of the maximal relaxation induced by each agonist to the maximal relaxation induced by (−)-isoprenaline (3 μM), the full agonist, in the absence of β-adrenoceptor antagonist. Statistical significance between two data sets was tested by Student's *t*-test. A P value of less than 0.05 was considered statistically significant.

2.5. Drugs

The following drugs were used: (−)-isoprenaline hydrochloride, (+)-isoprenaline (+)-bitartrate, (−)-noradrenaline (+)-bitartrate, (+)-noradrenaline (−)-bitartrate, (−)-adrenaline (+)-bitartrate, (±)-adrenaline hydrochloride, (−)-alprenolol (+)-tartrate, (±)-alprenolol hydrochloride, (±)-propranolol hydrochloride, (±)-nadolol, (±)-fenoterol hydrobromide, (±)-clenbuterol hydrochloride, (±)-salbutamol hemisulfate, (−)-phenylephrine hydrochloride, imipramine hydrochloride, normetanephrine hydrochloride, histamine dihydrochloride (Sigma–Aldrich, St. Louis, MO, USA); phentolamine mesylate (Novartis, Basel, Switzerland); BRL37344 [(*R**, *R**)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium] (Nacalaitesque, Kyoto, Japan); (±)-pindobind, (−)-pindolol, (±)-pindolol, (±)-CGP12177A hydrochloride ((±)-[4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-benzimidazol-2-one] hydrochloride), dopamine hydrochloride, (±)-dobutamine hydrochloride, (Research Biochemicals International, Natick, MA, USA) and (±)-bupranolol hydrochloride (Kaken Pharmaceutical, Tokyo, Japan). The other chemicals used were of analytical grade. (−)-Pindolol and (±)-pindolol were dissolved in 1 N HCl at a stock solution of 200 mM, respectively, and further diluted in distilled water. (±)-Pindobind was dissolved (20 μM) in dimethylsulfoxide. Final HCl and dimethylsulfoxide concentrations in the bath solution did not affect the relaxant responses. All other drugs were dissolved in distilled water.

3. Results

3.1. Stereoselectivity

The stereoisomers of catecholamines (isoprenaline, noradrenaline and adrenaline) and aryloxypropanolamines (alprenolol and pindolol) induced concentration-dependent relaxations of the guinea pig duodenum (Fig. 2). The pD_2 values and intrinsic activities of these drugs are summarized in Table 1. The (−)-enantiomers of isoprenaline and noradrenaline were more potent than its (+)-enantiomers

Table 1
Comparison of potencies and intrinsic activities for various drugs in the presence of (±)-propranolol (1 μM) and effects of (±)-pindobind (10 μM) at atypical β-adrenoceptors on the guinea pig duodenum

	n	Control		(±)-Pindobind-treated	
		pD_2 value	IA	pD_2 value	IA
(−)-Isoprenaline	10	6.43 ± 0.05	0.99 ± 0.01	5.89 ± 0.03	0.97 ± 0.01
(+)-Isoprenaline	10	4.88 ± 0.05	1.02 ± 0.02	NT[a]	
(−)-Noradrenaline	17	5.97 ± 0.04	0.82 ± 0.02	5.54 ± 0.05	0.88 ± 0.02
(+)-Noradrenaline	17	4.39 ± 0.03	0.83 ± 0.02	NT[a]	
(−)-Adrenaline	12	5.73 ± 0.06	0.79 ± 0.04	5.65 ± 0.06	0.81 ± 0.03
(±)-Adrenaline	12	5.40 ± 0.05	0.79 ± 0.03	NT[a]	
(−)-Alprenolol	11	6.43 ± 0.03	0.64 ± 0.02	NT[a]	
(±)-Alprenolol	11	6.15 ± 0.03	0.58 ± 0.03	NT[a]	
(−)-Pindolol	12	5.32 ± 0.05	0.83 ± 0.02	NT[a]	
(±)-Pindolol	12	5.05 ± 0.02	0.83 ± 0.04	NT[a]	
BRL37344	8	7.30 ± 0.02	0.89 ± 0.02	6.86 ± 0.04	0.65 ± 0.03[b]
(±)-CGP12177A	8	6.46 ± 0.02	0.91 ± 0.02	6.19 ± 0.08	0.70 ± 0.03[b]
(±)-Propranolol	6	not active up to 3 μM			
(±)-Bupranolol	6	not active up to 3 μM			
(±)-Nadolol	16	4.57 ± 0.04	0.44 ± 0.01	NT[a]	
(±)-Carteolol	9	4.90 ± 0.01[c]	0.93 ± 0.03[c]	NT[a]	
Dopamine	7	4.42 ± 0.04	0.54 ± 0.03	NT[a]	
(±)-Dobutamine	7	5.47 ± 0.05	0.88 ± 0.02	NT[a]	
(−)-Phenylephrine	7	4.35 ± 0.08	0.96 ± 0.04	NT[a]	
(±)-Salbutamol	8	5.34 ± 0.05	0.96 ± 0.02	NT[a]	
(±)-Clenbuterol	8	5.47 ± 0.04	1.07 ± 0.03	NT[a]	
(±)-Fenoterol	8	5.24 ± 0.05	0.95 ± 0.02	NT[a]	

Values are means ± S.E.M. of *n* experiments. IA: intrinsic activity.
[a] NT = not tested.
[b] $P < 0.05$, compared with its control.
[c] Values from Horinouchi and Koike, 2000.

($P < 0.05$), while the maximal relaxations induced by (−)-enantiomer and its (+)-enantiomer were not significantly different from each other ($P > 0.05$). Isomeric activity ratios ((+)/(−)) for enantiomer of isoprenaline and noradrenaline were 35.5- and 38.0-folds, respectively. Similarly, the (−)-isomers of adrenaline and aryloxypropanolamines were also more potent than its (±)-isomers while the maximal relaxations induced by (−)-isomers and its (±)-isomers were not significantly different from each other ($P > 0.05$). Isomeric activity ratios ((±)/(−)) for isomers of adrenaline, alprenolol and pindolol were 2.1-, 1.9- and 1.9-folds, respectively.

3.2. Effects of β-adrenoceptor alkylating agent (±)-pindobind

The concentration–response curves to catecholamines ((−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline) and to β_3-adrenoceptor agonists (BRL37344 and (±)-CGP12177A) were resistant to blockade by (±)-pindobind (10 μM) (Fig. 3, Table 1). (±)-Pindobind (10 μM) weakly shifted the concentration-dependent curves for the five drugs to the right by 3.5-, 2.7-, 1.2-, 2.8- and 1.5-folds, respectively. (±)-Pindobind induced a significant reduction in the maximum responses of BRL37344 and (±)-CGP12177A ($P < 0.05$), while the maximum responses of catecholamines were not affected by (±)-pindobind (Fig. 3, Table 1).

3.3. Structure–activity relationship of agonistic activity for atypical β-adrenoceptors

Dopamine, which lacks a β-hydroxyl group of catecholamines, was 35.5-fold less potent than (−)-noradrenaline and the maximal relaxant response of dopamine also was smaller than that of (−)-noradrenaline (Fig. 4A, Table 1). However, the dopamine derivative, (±)-dobutamine, which

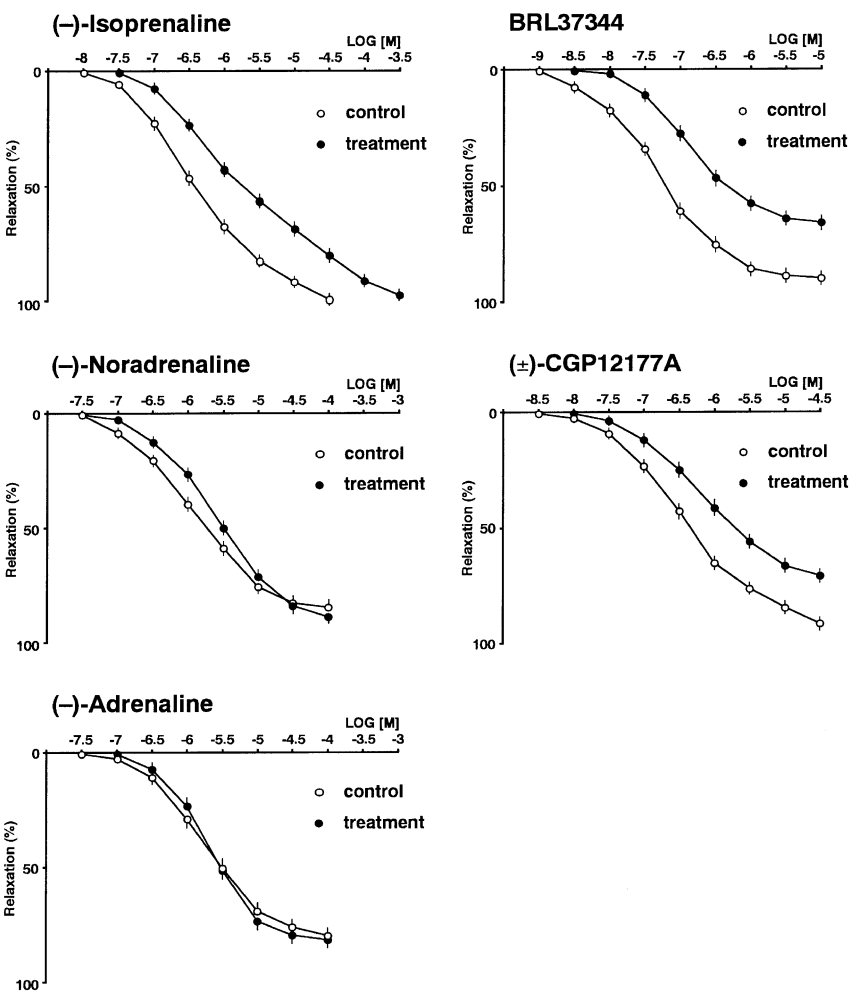

Fig. 3. Effects of (±)-pindobind (10 μM) on concentration–response curves for catecholamines ((−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline) and β_3-adrenoceptor agonists (BRL37344 and (±)-CGP12177A) in the presence of (±)-propranolol (1 μM). See Section 2 for tissue pretreatments. Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation (in the absence of (±)-propranolol (1 μM)) induced by (−)-isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E.M. of *n* experiments. The *n* values and the results are shown in Table 1.

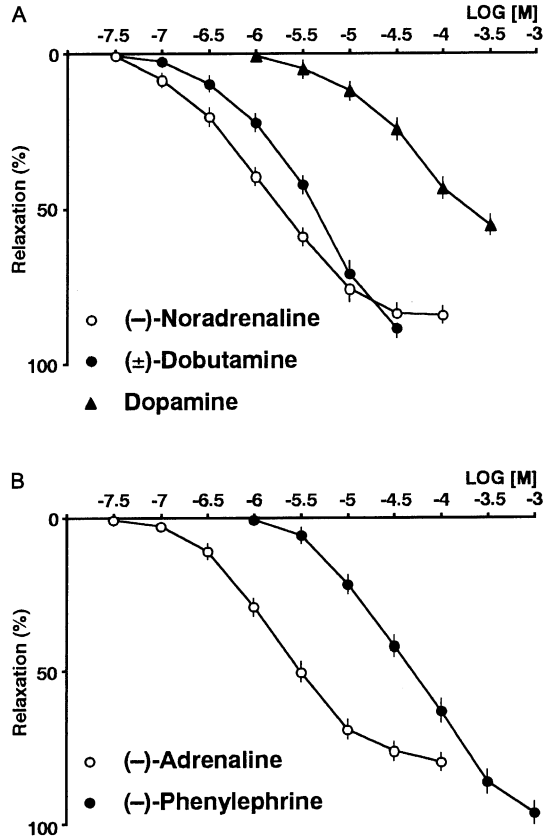

Fig. 4. Concentration–response curves for (−)-noradrenaline, dopamine, (±)-dobutamine, (−)-adrenaline and (−)-phenylephrine in the presence of (±)-propranolol (1 μM). Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation (in the absence of (±)-propranolol (1 μM)) induced by (−)-isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E.M. of *n* experiments. The *n* values and the results are shown in Table 1.

is structurally similar to the selective β_3-adrenoceptor agonist, BRL37344 (Fig. 1), was 11-fold more potent than dopamine and the maximal relaxant response of (±)-dobutamine was greater than that of dopamine (Fig. 4A, Table 1). (−)-Phenylephrine, which lacks a 4-hydroxyl group of (−)-adrenaline, was 24.0-fold less potent than (−)-adrenaline (Fig. 4B, Table 1).

3.4. Agonistic activities of selective β_2-adrenoceptor agonists on atypical β-adrenoceptors

The selective β_2-adrenoceptor agonists, (±)-fenoterol, (±)-clenbuterol and (±)-salbutamol, all caused graded relaxations in the guinea pig duodenum (Fig. 5, Table 1). The maximal relaxations of the three agonists were close to those of the full agonist, (−)-isoprenaline (Table 1).

3.5. Agonistic activities of aryloxypropanolamines on atypical β-adrenoceptors

The atypical β-adrenoceptor agonistic activities of aryloxypropanolamines were studied on the guinea pig duodenum in which β_1- and β_2-adrenoceptors were blocked. (±)-Propranolol, (±)-alprenolol and (±)-pindolol have the identical aryloxypropanolamine moiety having the *iso*-propyl substituent at the α position on the nitrogen substituent (Fig. 1). (±)-Bupranolol, (±)-nadolol and (±)-CGP12177A have the *tert*-butyl substituent at the α position on the nitrogen substituent on the identical aryloxypropanolamine moiety (Fig. 1). (±)-Propranolol and (±)-bupranolol lacked relaxant effects at concentrations

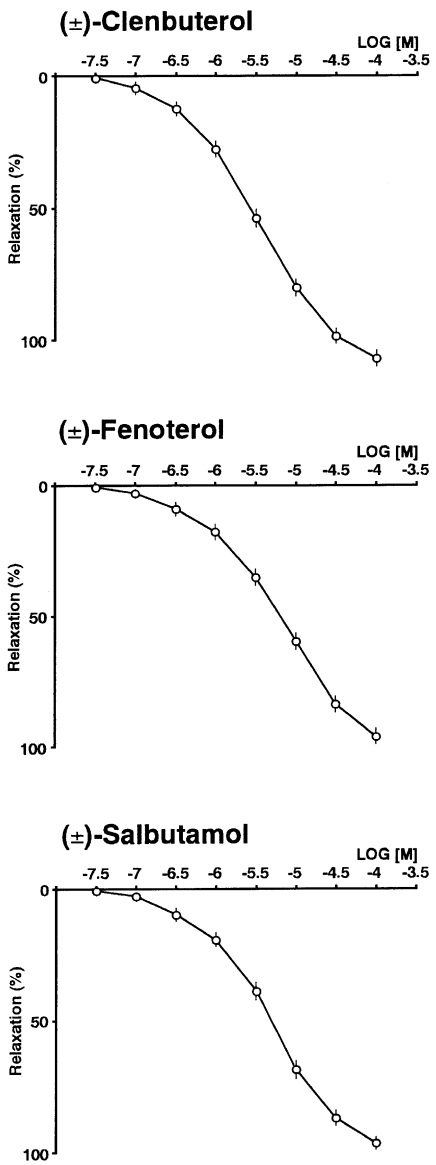

Fig. 5. Concentration–response curves for (±)-clenbuterol, (±)-salbutamol and (±)-fenoterol in the presence of (±)-propranolol (1 μM). Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation (in the absence of (±)-propranolol (1 μM)) induced by (−)-isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E.M. of *n* experiments. The *n* values and the results are shown in Table 1.

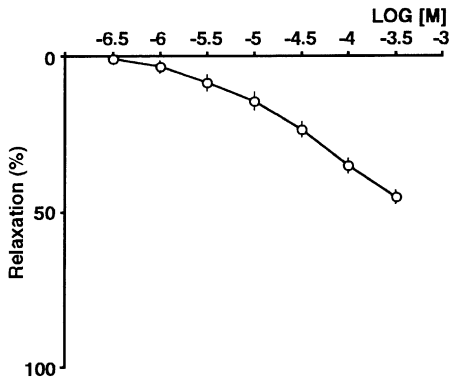

Fig. 6. Concentration–response curves for (±)-nadolol in the presence of (±)-propranolol (1 μM). Ordinate: relaxation (%), expressed as a percentage of the maximum relaxation (in the absence of (±)-propranolol (1 μM)) induced by (−)-isoprenaline (3 μM); abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E.M. of *n* experiments. The *n* values and the results are shown in Table 1.

up to 3 μM, while (±)-alprenolol, (±)-pindolol, (±)-nadolol and (±)-CGP12177A caused concentration-dependent relaxations ((Figs. 2, 3, 6), Table 1).

4. Discussion

Pharmacological characteristics for atypical β/β_3-adrenoceptors were described as follows: (i) high potency for a novel class of compounds, initially described as potent activators of lipolysis and thermogenesis in white and brown adipose tissues (e.g., BRL37344); (ii) partial agonist activities of several β_1- or β_2-adrenoceptor antagonists reflecting intrinsic sympathomimetic activities in heart tissue (e.g., CGP12177A); (iii) atypically low affinities and potencies for conventional β-adrenoceptor antagonists (e.g., propranolol); and (iv) atypically low stereoselectivity index for reference agonist and antagonist enantiomers as compared to those reported for traditional β_1- and β_2-adrenoceptors (Arch and Kaumann, 1993; Emorine et al., 1994). Previously, we have confirmed the above three criteria (i), (ii) and (iii) but not (iv) in the guinea pig duodenum (Horinouchi and Koike, 1999a). In the present study, we have carried out functional experiments to obtain the further characteristics of atypical β-adrenoceptors on the guinea pig duodenum.

Firstly, we examined the enantiomeric selectivity for the relaxant responses of catecholamines (isoprenaline, noradrenaline and adrenaline) and aryloxypropanolamines (alprenolol and pindolol) at atypical β-adrenoceptors on the guinea pig duodenum. The (−)-enantiomers of isoprenaline and noradrenaline were more potent than its (+)-enantiomers while the maximal relaxations induced by (−)-enantiomer and its (+)-enantiomer were not significantly different from each other (Fig. 2). The isomeric activity difference for (−)-isoprenaline and (+)-isoprenaline is 35.5-fold and that for (−)-noradrenaline and (+)-noradrenaline is 38-fold. However, isomeric activity ratios ((+)/(−)) for isomers of isoprenaline, noradrenaline and adrenaline from the guinea pig atria (β_1-adrenoceptors) were 1023-, 501- and 63-folds, respectively, and those from the guinea pig trachea (β_2-adrenoceptors) were 490-, 200- and 29-folds, respectively (Buckner and Patil, 1971). These results suggest that the stereoselectivity of atypical β-adrenoceptors is lower than that of β_1- and β_2-adrenoceptors on the guinea pig.

However, the isomeric activity ratio ((+)/(−)) of trimetoquinol, which is the selective β_2-adrenoceptor agonist, is greater on atypical β-adrenoceptors than on β_1- and β_2-adrenoceptors on rat (Lezama et al., 1996). Furthermore, the (−)-enantiomer of trimetoquinol was more potent than its (+)-enantiomer on atypical β-adrenoceptors of rat esophageal smooth muscle (Lezama et al., 1996). In contrast, the bronchorelaxant activity of (+)-enantiomer for salbutamol (the selective β_2-adrenoceptor agonist) was 68-fold more potent than its (−)-enantiomer (Brittain et al., 1973). Thus, the relative activity between (−)-enantiomer and its (+)-enantiomer may depend on β-adrenoceptor subtypes or β-adrenoceptor agonists.

(±)-Isomer is itself a 50:50 mixture of (−)-isomer and (+)-isomer. The dissociation between pharmacological activity of (−)-isomer and (+)-isomer was observed with rasemic pindolol (Walter et al., 1984). The agonistic and antagonistic activities of (+)-pindolol were mediated through sinoatrial β_2-adrenoceptors, whereas (−)-pindolol causes stimulant effects through atrial receptors distinct from β_1- and β_2-adrenoceptors in the guinea pig (Walter et al., 1984; Kaumann, 1989). In the present study, isomeric activity ratios ((±)/(−)) for isomers of adrenaline, alprenolol and pindolol were approximately twofold, indicating that the relaxant effects of (±)-isomers are due to the pharmacological activity of the (−)-enantiomers, and that (+)-enantiomers are inactive at concentrations using in this study. Thus, the atypical β-adrenoceptor-mediated effects of (−)-enantiomers may occur at doses lower than those used in both (+)-enantiomers and racemic (±)-isomers.

The relaxant effects to catecholamines ((−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline) and to β_3-adrenoceptor agonists (BRL37344 and (±)-CGP12177A) were resistant to blockade by (±)-pindobind (10 μM), the β-adrenoceptor alkylating agent. Lezama et al. (1996) have reported that (−)-isoprenaline-induced relaxations were resistant to alkylation by (±)-pindobind at atypical β-adrenoceptors on rat esophageal smooth muscle. However, (±)-pindobind shifted the concentration–response curves for (−)-isoprenaline to the right by 606 ± 247-fold in the guinea pig left atria (β_1-adrenoceptors) and 300 ± 150-fold in the guinea pig trachea (β_2-adrenoceptors), respectively, and induced reductions in the maximal relaxant responses (Molenaar et al., 1988). These results indicate that phar-

macological properties of atypical β-adrenoceptors in duodenum, which were resistant to alkylation by (±)-pindobind, differ from β_1-adrenoceptors in left atria and β_2-adrenoceptors in trachea on the guinea pig tissues.

(±)-Pindobind induced a significant reduction in the maximum relaxations to β_3-adrenoceptor agonists (BRL37344 and (±)-CGP12177A) while those to catecholamines ((−)-isoprenaline, (−)-noradrenaline and (−)-adrenaline) were not affected by (±)-pindobind (Fig. 3). However, we have shown that the relaxant responses to the five agonists were competitively antagonized by non-selective β_1-, β_2- and β_3-adrenoceptor antagonist, (±)-bupranolol, without reducing the maximum relaxations in the guinea pig duodenum (Horinouchi and Koike, 1999a). These results suggested that the spare receptors were quantitatively abundant on atypical β-adrenoceptors in this tissue. Therefore, when explained, the reduction in the maximum relaxations to β_3-adrenoceptor agonists by (±)-pindobind, it is unlikely that the spare receptors are an important factor, but it is possible that response-coupling-efficiency between catecholamines and β_3-adrenoceptor agonists (non-catecholamine agonists) may differ in this system.

The β-adrenoceptors are hybrid, with the interaction site for the aromatic moiety of aryloxypropanolamines (mainly antagonists) or arylethanolamines (mainly agonists) having β_2-adrenoceptor characteristics, and the interaction site for the nitrogen substituent having β_1-adrenoceptor characteristics (Harms et al., 1974). The selective β_3-adrenoceptor agonist, BRL37344, belongs to arylethanolamine class and (±)-CGP12177A, which belongs to aryloxypropanolamine class, possesses both β_1- and β_2-adrenoceptor antagonistic activities and atypical β/β_3-adrenoceptor agonistic activities. Furthermore, many compounds, which have aryloxypropanolamine or arylethanolamine moiety, exhibit atypical β/β_3-adrenoceptor agonistic effects (Arch and Kaumann, 1993). We therefore have carried out functional experiments to clarify whether the following drugs possess atypical β-adrenoceptor agonistic activities: arylethanolamines (catecholamines, selective β_2-adrenoceptor agonists and BRL37344), their derivatives (dopamine, (±)-dobutamine and (−)-phenylephrine) and a variety of aryloxypropanolamines (Fig. 1).

(−)-Isoprenaline, (−)-noradrenaline and (−)-adrenaline have three hydroxyl groups substituted at positions 3 and 4 of benzene ring and at β position on the nitrogen substituent. Dopamine lacks a β-hydroxyl group on the nitrogen substituent essential for the agonistic activity of catecholamines including neurohumoral transmitter, (−)-noradrenaline. (−)-Phenylephrine differs from (−)-adrenaline, only lacking a hydroxyl group at 4-position on the benzene ring. (−)-Noradrenaline and (−)-adrenaline were more potent than dopamine and (−)-phenylephrine, respectively, indicating that the β-hydroxyl group or 4-hydroxyl group substituent characterized efficacy. In addition, this modification creates an asymmetrical center, leading to isomerization of the molecule, and this polar β-hydroxyl group may interact with an electrophilic center and form a hydrogen bond with an amino acid chain inside the receptor groove. (±)-Dobutamine resembles dopamine structurally but possesses a bulky aromatic substituent on the amino group. In addition, the chemical structure of (±)-dobutamine is similar to BRL37344, which also have a bulky group on the alkylamine chain. The groups increase steric bulk and lipophilicity at the end of the alkylamine chain. (±)-Dobutamine was 11-fold more potent than dopamine, suggesting that increase in the size of the end of the alkylamine chain increases atypical β-adrenoceptor agonistic activity without inducing the steric hindrance in the guinea pig duodenum. These facts were in agreement with those reported by Blin et al. (1993) for Chinese hamster ovary cells expressing the human β_3-adrenoceptors. Furthermore, Ainsworth and Smith (1980) have demonstrated that both the β-hydroxyl group and the methyl substituent at the α position on the nitrogen substituent are essential for potent activity at atypical β/β_3-adrenoceptors. Thus, the nor-hydroxyl compound and the nor-methyl compounds had very significantly reduced activity relative to BRL37344. In general, maximal β-adrenoceptor activity depends on the presence of hydroxyl groups on positions 3 and 4 of the benzene ring (Hardman et al., 1996). When one or both of these groups are absent, with no other aromatic substitution, the overall potency is reduced. Furthermore, studies of human β_3-adrenoceptors based on computer modelling suggested that the hydroxyl groups on serine residues 209 and 212 probably form hydrogen bonds with the catechol hydroxyl groups at positions 3 and 4, respectively (Strosberg and Piétri-Rouxel, 1996). However, BRL37344 has the 3-chlorine atom at the aromatic ring of the arylethanolamine moiety, whereas its atypical β-adrenoceptor agonistic activity is the most potent in the present study. Hoey et al. (1996b) synthesized cyanopindolol and its analogues and tested the agonistic and antagonistic activities for these compounds at β_3-adrenoceptors in rat ileum. The replacement of hydrogen with the iodine atom at the indole 2-position, e.g., changing cyanopindolol to iodocyanopindolol, resulted in an increase in agonistic potency from the pD_2 value of 5.28 to the value of 7.00 (Hoey et al., 1996b). In contrast, (±)-bupranolol, which has the chlorine atom at the position 2 on the benzene ring, possesses atypical β/β_3-adrenoceptor antagonistic activity without agonistic effects (Kaumann, 1989; Horinouchi and Koike, 1999a, b). BRL37344 and (±)-bupranolol have a structure close to that of (−)-isoprenaline, except that both hydroxyl groups of the phenyl moiety are substituted by an inductor-donor chlorine atom, which favors delocalization of benzenic π-electrons and may increase hydrophilicity. These results suggested that the halogen moiety at the benzene ring is important to interact with atypical β-adrenoceptors but could not clearly characterize the agonistic and antagonistic effects for drugs at atypical β-adrenoceptors.

Chemically, characterization of selective β_2-adrenoceptor agonists ((±)-fenoterol, (±)-clenbuterol and (±)-salbutamol) is described as follows: modifications have included placing the hydroxyl groups at positions 3 and 5 of the phenyl ring or substituting another moiety for the hydroxyl group at position 3. These drugs induced relaxations of the guinea pig duodenum in which β_1- and β_2-adrenoceptors were blocked. These results suggested that selective β_2-adrenoceptor agonists also possess agonistic activities at atypical β-adrenoceptors.

Several β-adrenoceptor antagonists show intrinsic activity at concentrations greatly exceeding those required for β-adrenoceptor blockade on the heart (Kaumann, 1989). Furthermore, it is well established that common structural requirements characterized the selective or potent β_3-adrenoceptor ligands, i.e., a 18–20 carbon backbone length, an aromatic ring (substituted or not), and an (oxy)hydroxylalkylamine chain ending in an indol function or a phenyl carrying hydroxyl, ether, or acid functions, which increase steric bulk and moderate lipophilicity (Blin et al., 1993). (±)-Propranolol, (±)-alprenolol, (±)-pindolol, (±)-bupranolol, (±)-nadolol, (±)-CGP12177A and (±)-carteolol belong to aryloxypropanolamine. The first three drugs have the *iso*-propyl substituent and the last four have the *tert*-butyl substituent at the α position on the nitrogen substituent on side chain (Fig. 1). (±)-Propranolol and (±)-bupranolol had no agonistic effects, whereas the others produced concentration-dependent relaxations on atypical β-adrenoceptors in the guinea pig duodenum. These results suggested that it is unlikely for the aryloxypropanolamine side chains to be the primary determinant of the agonistic activities at atypical β-adrenoceptors in the guinea pig duodenum. The structural requirements for agonistic or antagonistic effects may be characterized by the substituent of aromatic groups of the aryloxypropanolamines at atypical β-adrenoceptors in this tissue. Furthermore, Blin et al. (1993) have reported that (±)-propranolol, (±)-alprenolol, (±)-pindolol (±)-nadolol and (±)-CGP12177A behave as β_1- and β_2-adrenoceptor antagonists and β_3-adrenoceptor agonist, whereas (±)-bupranolol acts as β_1-, β_2- and β_3-adrenoceptor antagonist in Chinese hamster ovary cells expressing the human β_3-adrenoceptors. It is possible that pharmacological properties of the guinea pig atypical β-adrenoceptors are similar to those of the human β_3-adrenoceptors. However, site-directed mutagenesis studies based on computer modelling have not yet explained why some β_1- or β_2-adrenoceptor antagonists behave as atypical β-adrenoceptor agonists (Strosberg, 1993, 1995; Blin et al., 1995; Gros et al., 1998).

In conclusion, our present results clearly demonstrated that pharmacological properties at atypical β-adrenoceptors differ from that at β_1- and β_2-adrenoceptors on the guinea pig. In addition, we also provided further atypical β-adrenoceptors characteristics. Thus, pharmacological studies on atypical β-adrenoceptors, using various of β-adrenoceptor-related drugs with differing β-adrenoceptor profiles, are important in the development of new atypical β-adrenoceptor drugs.

Acknowledgements

We thank Kaken Pharmaceutical (Tokyo, Japan) for generously providing (±)-bupranolol hydrochloride.

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