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Short communication

Agonistic activity of SR59230A at atypical β-adrenoceptors in guinea pig gastric fundus and duodenum

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Abstract

We have recently suggested that atypical β-adrenoceptors are present in guinea pig gastric fundus and duodenum. In the present study, we have shown that SR59230A (3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2*S*)-2-propanol oxalate), a selective β_3-adrenoceptor antagonist, possesses agonistic activities at atypical β-adrenoceptors in these tissues. SR59230A caused concentration-dependent relaxations. However, (±)-propranolol (1 μM) did not affect SR59230A-induced relaxations. Pretreatment of with a combination of (±)-propranolol (1 μM) and the non-selective β_1-, β_2-, β_3- and β_4-adrenoceptor antagonist, (±)-bupranolol (30 μM), significantly antagonized the relaxant effects induced by SR59230A. The results clearly indicate that SR59230A acts as an atypical β-adrenoceptor agonist on guinea pig gastric fundus and duodenum.

Keywords: SR59230A; (±)-Bupranolol; β-Adrenoceptor, atypical; β_3-Adrenoceptor; Gastric fundus, guinea pig; Duodenum, guinea pig

1. Introduction

SR59230A (3-(2-ethylphenoxy)-1-[(1*S*)-1,2,3,4-tetrahydronaphth-1-ylamino]-(2*S*)-2-propanol oxalate), an aryloxypropanolaminotetralin, has been developed as the first selective β_3-adrenoceptor antagonist for the gut (Manara et al., 1995), while the phenylethanolaminotetralins, like SR58611A (*N*[(2*S*)-7-carbethoxymethoxy-1,2,3,4-tetrahydronaphth-2-yl]-(2*R*)-2-hydroxy-2-(3-chlorophenyl)-ethanamine hydrochloride), possess atypical β/β_3-adrenoceptor agonist properties which are antagonized by SR59230A (Bianchetti and Manara, 1990; Croci et al., 1995). It is possible that, depending on the species, the aminotetralin moiety of SR59230A recognizes the similar atypical β/β_3-adrenoceptor of phenylethylaminotetralin. In addition, SR59230A has a bulky group, like ICI D7114 ((*S*)-4-[2-hydroxy-3-phenoxy-propylamino-ethoxy]-*N*-(2-methoxyethyl) phenoxyacetamide) which possesses atypical β/β_3-adrenoceptor partial agonist effect in the guinea pig ileum (Growcott et al., 1992, 1993b) and antagonist activity in rat ileum (Growcott et al., 1992, 1993a,b). Therefore, in order to investigate the agonist effects of SR59230A at atypical β-adrenoceptors in guinea pig tissues, we have carried out functional experiments in the gastric fundus and duodenum according to the method of Horinouchi and Koike (1999a,b).

2. Methods

2.1. Mechanical responses

Experiments were carried out on isolated longitudinal smooth muscle strip of guinea pig gastric fundus and duodenum. The gastric fundus and duodenum were isolated from male guinea pigs weighing 300–500 g, which were killed by cervical dislocation. After the mucosae was removed, strips of approximately 15–20 mm in length and 4–6 mm in width were cut in the direction of the longitudinal smooth muscle, with a maximum of four strips from one guinea pig gastric fundus. After careful flushing of the luminal contents, the outer layer of duodenum (approximately 10–15 mm in length) containing longitudinal smooth muscle was carefully removed with a cotton swab. Strips were mounted vertically under an initial tension of 0.5 g in a 20-ml organ bath containing Ringer–Locke solution (in mM: NaCl 154; KCl 5.6; $CaCl_2$ 2.2; $MgCl_2$

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Table 1
Potencies (pD_2) and intrinsic activities (IA) of SR59230A and effects of antagonists at atypical β-adrenoceptors on the guinea pig gastric fundus and duodenum

	n	pD_2 value	IA
Gastric fundus			
Control	9	7.10 ± 0.04	0.78 ± 0.03
(±)-Propranolol (1 μM)	8	7.08 ± 0.02	0.79 ± 0.03
(±)-Propranolol (1 μM) + (±)-bupranolol (30 μM)	7	6.20 ± 0.04	0.77 ± 0.04
Duodenum			
Control	9	6.49 ± 0.04	0.83 ± 0.03
(±)-Propranolol (1 μM)	8	6.41 ± 0.05	0.84 ± 0.03
(±)-Propranolol (1 μM) + (±)-bupranolol (30 μM)	6	5.62 ± 0.04	0.79 ± 0.04

Data are means ± S.E.M. from *n* experiments. pD_2 value = $-\log EC_{50}$ (EC_{50}: concentration of drug inducing half-maximum relaxation). IA: intrinsic activity (maximum relaxation of SR59230A with respect to the maximum relaxation induced by the full agonist (−)-isoprenaline (3 μM) in the absence of β-adrenoceptor antagonist).

2.1; $NaHCO_3$ 5.9; and glucose 2.8), maintained at 32°C and bubbled with a mixture of 95% O_2 and 5% CO_2. Desmethylimipramine (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 catecholamine) were present in the medium throughout all experiments. To measure relaxations, strips were contracted with prostaglandin $F_{2\alpha}$ (gastric fundus) or histamine (duodenum) and were then exposed to increasing half-log cumulative concentrations of β-adrenoceptor agonists. The relaxant effect induced by SR59230A was expressed as a percentage of the maximal relaxation produced by (−)-isoprenaline (3 μM), the reference drug, in the absence of β-adrenoceptor antagonist. When (±)-propranolol (1 μM) and (±)-bupranolol (30 μM) were used, each drug was added to the bath and allowed to equilibrate with the tissue for 30 min before addition of prostaglandin $F_{2\alpha}$ or histamine. A single concentration–response curve to SR59230A was determined for each strip in the absence of β-adrenoceptor antagonist, in the presence of (±)-propranolol (1 μM) and/or in the presence of both (±)-propranolol (1 μM) and (±)-bupranolol (30 μM). An apparent pA_2 value for (±)-bupranolol was calculated by the equation, apparent $pA_2 = \log(CR - 1) - \log[B]$ (Van Rossum, 1963), where [B] is the molar concentration of (±)-bupranolol and CR is the ratio of EC_{50} values in the presence and absence of β-adrenoceptor antagonist.

2.2. Data analysis

The results are expressed as means ± S.E.M. of the number (*n*) of experiments. 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.

2.3. Drugs

The drugs used were obtained from the following sources: (−)-isoprenaline hydrochloride, (±)-propranolol hydrochloride, histamine dihydrochloride, desmethylimipramine hydrochloride, normetanephrine hydrochloride (Sigma, St. Louis, MO, USA); (±)-bupranolol hydrochloride was kindly supplied by Kaken Pharmaceutical (Tokyo, Japan); phentolamine mesylate (Novartis, Basel, Switzerland) and prostaglandin $F_{2\alpha}$ (Ono Pharmaceutical, Osaka, Japan). SR59230A was synthesized in our laboratory. SR59230A was dissolved in dimethylsulfoxide at a stock solution of 20 mM and further diluted in distilled water. All other drugs were dissolved in distilled water. The other chemicals used were of analytical grade.

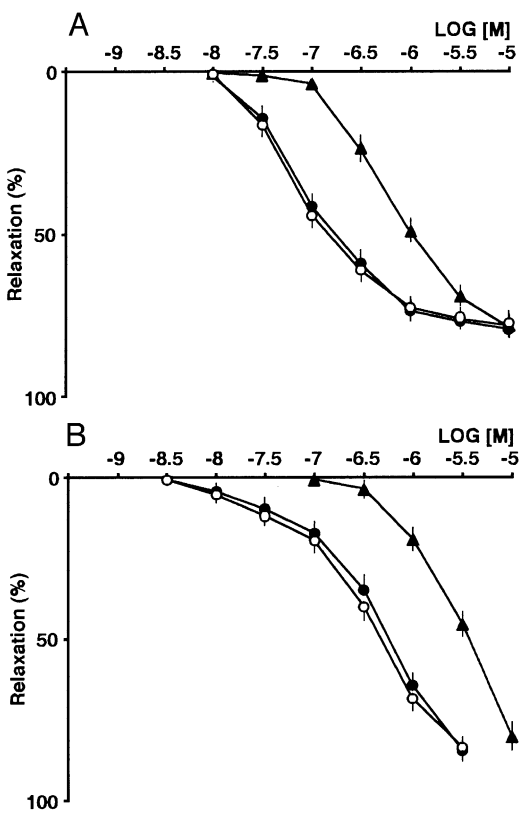

Fig. 1. The effect of (±)-propranolol and (±)-bupranolol on SR59230A-induced relaxation in prostaglandin $F_{2\alpha}$ (3 μM)-precontracted gastric fundus (A) and histamine (10 μM)-precontracted duodenum (B) of the guinea pig. Control, no (±)-propranolol and (±)-bupranolol (○); (±)-propranolol 1 μM (●); (±)-propranolol 1 μM plus (±)-bupranolol 30 μM (▲). Ordinate: relaxation (%), expressed as a percentage relative to the maximum relaxation induced by (−)-isoprenaline (3 μM) in the absence of β-adrenoceptor antagonist, abscissa: concentration (*M*) of SR59230A. Each point represents the mean ± S.E.M. of 6–9 experiments.

3. Results

In the absence of β-adrenoceptor antagonist, SR59230A induced concentration-dependent relaxations of precontracted guinea pig gastric fundus and duodenum (Fig. 1A,B, Table 1). The relaxant responses of SR59230A were resistant to blockade with the non-selective β_1- and β_2-adrenoceptor antagonist, (±)-propranolol (1 μM, Fig. 1A,B, Table 1). However, a combination of (±)-propranolol (1 μM) and the non-selective β_1-, β_2-, β_3- and β_4-adrenoceptor antagonist, (±)-bupranolol (30 μM), caused a rightward shift of the concentration–response curve to SR59230A (Fig. 1A,B, Table 1). The apparent pA_2 values for (±)-bupranolol against SR59230A were 5.36 ± 0.04 ($n = 7$) in the guinea pig gastric fundus and 5.23 ± 0.07 ($n = 6$) in the guinea pig duodenum, respectively.

4. Discussion

SR59230A has been generally studied as the novel selective β_3-adrenoceptor antagonist. SR59230A and the novel β_3-adrenoceptor agonist, SR58611A, have an aminotetralin group in their chemical structure (Nisoli et al., 1994). In addition, many selective β_3-adrenoceptor agonists (e.g., BRL37344 ((*R**,*R**)-(±)-4-[2-[(2-(3-chlorophenyl)-2-hydroxyethyl)amino]propyl]phenoxyacetic acid sodium) and ICI D7114) have a bulky group on the arylethanolamine or aryloxypropanolamine side chain (Arch and Kaumann, 1993). Especially, ICI D7114 has been synthesized as a selective atypical β/β_3-adrenoceptor agonist (Holloway et al., 1991), and then the atypical β/β_3-adrenoceptor antagonistic effects of ICI D7114 were reported (Growcott et al., 1992, 1993a). Furthermore, (±)-propranolol, (±)-bupranolol, (±)-CGP12177A ((±)-[4-[3-[(1,1-dimethylethyl)amino]-2-hydroxypropoxy]-1,3-dihydro-2*H*-benzimidazol-2-one] hydrochloride), (±)-carteolol and SR59230A belong to aryloxypropanolamine class. The two former had no agonistic effect at concentration up to 1 μM (data not shown), whereas (±)-CGP12177A and (±)-carteolol also behave as an agonist at atypical β-adrenoceptors in the guinea pig gastric fundus and duodenum (Horinouchi and Koike, 1999a,b, 2000a,b). We, therefore, considered that SR59230A also possesses the agonist activity at atypical β/β_3-adrenoceptors. We now demonstrate that SR59230A acts as an 'atypical β-adrenoceptor agonist' at atypical β-adrenoceptors in the guinea pig gastric fundus and duodenum. SR59230A caused graded relaxations of these tissues. The non-selective β_1- and β_2-adrenoceptor antagonist, (±)-propranolol (1 μM), had no effect on SR59230A-induced relaxation, indicating that β_1- and β_2-adrenoceptors did not play a role in the relaxant effects of SR59230A. In the presence of (±)-propranolol (1 μM) to block β_1- and β_2-adrenoceptor, however, the non-selective β_1-, β_2-, β_3- and β_4-adrenoceptor antagonist, (±)-bupranolol (30 μM), blocked the relaxant responses of SR59230A in both the guinea pig gastric fundus and duodenum, thus, suggesting a involvement of either β_3- or β_4/atypical β-adrenoceptor for SR59230A mediated relaxation. The low pA_2 values for (±)-bupranolol of 5.36 in the gastric fundus and 5.23 in the duodenum were close to the values (5.29; gastric fundus, 5.31; duodenum) against (±)-carteolol obtained in atypical β-adrenoceptors on the guinea pig (Horinouchi and Koike, 2000a,b) and the values (5.0–5.2) against (±)-CGP12177A obtained in the rat aorta atypical β-adrenoceptors which were similar to putative β_4-adrenoceptors (Brawley et al., 2000). Furthermore, the pA_2 values for (±)-bupranolol against SR59230A are lower than reported by Kaumann (1989) for β_1- and β_2-adrenoceptors. (±)-Bupranolol is a potent β_1- and β_2-adrenoceptor antagonist with weaker antagonistic activity at atypical β/β_3-adrenoceptor (Kaumann and Molenaar, 1996; Malinowska and Schlicker, 1997; Horinouchi and Koike, 1999a,b) and β_4-adrenoceptor (Kaumann, 1996; Kaumann and Molenaar, 1996; Malinowska and Schlicker, 1996, 1997). In addition, SR59230A is the potent selective β_3-adrenoceptor antagonist itself, indicating the agonistic effects of SR59230A may be mediated by further atypical β-adrenoceptor subtypes (e.g. putative β_4-adrenoceptor proposed by Sarsero et al., 1998) different from β_3-adrenoceptors stimulating by BRL37344, the selective β_3-adrenoceptor agonist, in the guinea pig gastric fundus and duodenum. Furthermore, these facts suggest that (±)-bupranolol acts as a non-selective atypical β-adrenoceptor antagonist.

In conclusion, SR59230A possesses agonistic activities at atypical β-adrenoceptors in the guinea pig gastric fundus and duodenum. It is possible that the compounds, which have a bulky group (the aminotetralin moiety in SR59230A's case) on ethanolamine or propanolamine side chain, have atypical β-adrenoceptor characteristics in the guinea pig gastrointestinal tissue systems. Furthermore, agonist or antagonist properties of SR59230A may depend on receptor differences in animal species.

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