Structure-Activity Relationship Studies of (±)-Terbutaline and (±)-Fenoterol on β3-Adrenoceptors in the Guinea Pig Gastric Fundus

Takahiro HORINOUCHI1, Yoshiko NAKAGAWA1, Makiko WAKABAYASHI1 and Katsuo KOIKE1

1Department of Chemical Pharmacology, Toho University, School of Pharmaceutical Sciences, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan

Abstract

(±)-Terbutaline and (±)-fenoterol are both arylethanolamine analogs that have tert-butyl and aryliso-propyl substituents respectively at the α position on the nitrogen of the ethanolamine side chain. In the present study, we have investigated the structure-activity relationships of (±)-terbutaline and (±)-fenoterol as β3-adrenoceptor agonists in the guinea pig gastric fundus. (±)-Terbutaline and (±)-fenoterol induced concentration-dependent relaxation of the precontracted gastric fundus with pD2 values of 4.45 ± 0.10 and 5.90 ± 0.09, and intrinsic activities of 1.00 ± 0.03 and 0.99 ± 0.01 respectively. The combination of the selective β1-adrenoceptor antagonist (±)-atenolol (100 µM), and the selective β2-adrenoceptor antagonist (±)-butoxamine (100 µM), produced a 2 and 6 fold rightward shift of the concentration-response curves for (±)-terbutaline and (±)-fenoterol respectively, without depressing the maximal responses. The order of potency of these agonists was (pD2 value): (±)-fenoterol (5.09 ± 0.10) > (±)-terbutaline (4.13 ± 0.08). In the presence of (±)-atenolol and (±)-butoxamine, however, the non-selective β1, β2 and β3-adrenoceptor antagonist (±)-bupranolol caused a concentration-dependent rightward shift of the concentration-response curves for (±)-terbutaline and (±)-fenoterol. Schild plot analyses of the effects of (±)-bupranolol against these agonists gave pA2 values of 6.21 ± 0.07 ((±)-terbutaline) and 6.37 ± 0.06 ((±)-fenoterol) respectively, and the slopes of the Schild plot were not significantly different from unity (p>0.05). These results suggest that the relaxant responses to (±)-terbutaline and (±)-fenoterol are mainly mediated through β3-adrenoceptors in the guinea pig gastric fundus. The β3-adrenoceptor agonist potencies of arylethanolamine analogs depend on the size of the end of the alkylamine side chain.

Key words: atypical β-adrenoceptor, β3-adrenoceptor, structure-activity relationship, β2-adrenoceptor agonist, Guinea pig gastric fundus
Introduction

β-Adrenoceptors were initially classified into β₁- and β₂-adrenoceptor subtypes by Lands et al. (1967a; 1967b) based on the relative potencies of sympathomimetic amines and tissue localization. Functional and molecular cloning studies have indicated the presence of atypical β-adrenoceptors or β₃-adrenoceptors, that differ from the classical β₁- and β₂-adrenoceptors (for review see Arch and Kaumann, 1993). Atypical β-adrenoceptors, including β₃-adrenoceptors, mediated relaxation in gastrointestinal smooth muscle from a variety of species including guinea pig, rat, rabbit, and man (for review see Manara et al., 1995b). The responses mediated through β₃-adrenoceptors are characterized by the following four criteria: (i) low sensitivity to classical β₁- and β₂-adrenoceptors antagonists (e.g., propranolol), (ii) stimulation by selective β₃-adrenoceptor agonists (e.g., BRL37344), (iii) stimulation by non-conventional partial β₃-adrenoceptor agonists (e.g., CGP12177A), and (iv) sensitivity to either selective β₃-adrenoceptor antagonists (e.g., SR59230A) (Arch and Kaumann, 1993; Manara et al., 1995a; Kaumann and Molenaar, 1996) or the non-selective β₁-, β₂-, and β₃-adrenoceptor antagonists (e.g., bupranolol) (Kaumann, 1989).

In the guinea pig gastric fundus, Coleman et al. (1987) reported that relaxant responses to isoprenaline and noradrenaline were resistant to propranolol. Recently, we showed that β-adrenoceptors of the guinea pig gastric fundus fulfill all four criteria (Horinouchi and Koike, 1999; Horinouchi et al., 2001d) and we described such β-adrenoceptors as ‘β₃-adrenoceptors’. Furthermore, ary lethanalamines (e.g., (-)-isoprenaline and BRL37344) and aryloxypropanolamines (e.g., (±)-CGP12177A, (±)-pindolol, (±)-carteolol and SR59230A) produced β₃-adrenoceptor mediating relaxation in the guinea pig gastric fundus (Horinouchi and Koike, 1999; 2000; 2001a; 2001b).

(±)-Terbutaline and (±)-fenoterol, selective β₂-adrenoceptor agonists, have tert-butyl and aryliso-propyl substituents respectively at the α position on the nitrogen of the ethanolamine side chain (see Fig. 1 for chemical structure). These drugs have an asymmetric carbon (Fig. 1). A mixture of equal parts of its stereoisomers is used for the treatment of bronchial asthma and in animal experiments. In addition, (±)-terbutaline and (±)-fenoterol bear a structural resemblance to (-)-isoprenaline and BRL37344 respectively. Therefore, we considered that (±)-terbutaline and (±)-fenoterol could also possess β₂-adrenoceptor agonistic activity in the guinea pig gastric fundus.

Fig. 1. Chemical structures of terbutaline and fenoterol used in the present study. The presence of the asymmetric carbon atom is denoted by *.
The aim of the present study was to clarify whether the relaxant responses to (±)-terbutaline and (±)-fenoterol are mediated by β3-adrenoceptors in the guinea pig gastric fundus, and to establish the chemical requirements for β3-adrenoceptor agonists by comparing the potencies of (±)-terbutaline and (±)-fenoterol.

Materials and Methods

Animals and tissue preparation

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

Guinea pigs were sacrificed and the gastric fundus was isolated. The stomach contents were removed immediately and the connective tissue was dissected away. The gastric fundus was opened and cut into strips (4–6 mm wide, 15–20 mm long) running parallel to the longitudinal smooth muscle fibers and the gastric mucosae was carefully removed from the muscle layer. Strips 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; CaCl2, 2.2; MgCl2, 2.1; NaHCO3, 5.9 and glucose, 2.8 mM), maintained at 32°C and bubbled continuously with a mixture of 95% O2 and 5% CO2. Imipramine (1 μM, a neuronal uptake inhibitor), normetanephrine (10 μM, an extraneuronal uptake inhibitor), phentolamine (10 μM, an α-adrenoceptor antagonist) and L-ascorbic acid (10 μM, to prevent oxidation of catecholamine) were present in the medium throughout all experiments.

Experimental protocols

After the preparations were allowed to equilibrate for 30 min in the absence of β-adrenoceptor antagonist, the preparations were contracted with prostaglandin F2α (PGF2α; 3 μM), which induced a contraction equal to 70–80% of the maximal PGF2α-induced contraction. A sustained plateau phase was observed approximately 30 min after the addition of PGF2α, then test drugs were added cumulatively. The β-adrenoceptor-mediated relaxations caused by the test drugs were determined by measuring the inhibition of the PGF2α-induced contraction. Firstly, concentration-response curves for (−)-isoprenaline (up to 3 μM) were generated as controls (100%). PGF2α (3 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. 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 antagonistic effects of the combination of (±)-atenolol, (±)-butoxamine
and (±)-bupranolol, each antagonist was added to the bath 30 min before the addition of PGF$_{2\alpha}$. (±)-Atenolol (100 µM; an aryloxypropanolamine analog), (±)-butoxamine (100 µM; an arylethanolamine analog) and (±)-bupranolol (≤10 µM; an aryloxypropanolamine analog) themselves did not induce the inhibition of the PGF$_{2\alpha}$-induced contraction (data not shown).

Data analysis

The results are expressed as the mean ± S.E.M. for the number (n) of experiments performed. Agonistic potency was expressed 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 a β-adrenoceptor antagonist. The competitive antagonistic potency of (±)-bupranolol was 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). Statistical significance between two data sets was tested by the Student’s t test. A P value of less than 0.05 was considered to be statistically significant.

Drugs

The following drugs were used: (−)-isoprenaline hydrochloride, (±)-terbutaline hemisulfate, (±)-fenoterol hydrobromide, imipramine hydrochloride, normetanephrine hydrochloride, (±)-butoxamine hydrochloride, L-ascorbic acid (Sigma-Aldrich Co., St. Louis, Mo., USA); phentolamine mesylate (Novartis, Basel, Switzerland); (±)-atenolol (Research Biochemicals International, Natick, Mass., USA); (±)-bupranolol hydrochloride (Kaken Pharmaceutical Co., Ltd, Tokyo, Japan) and prostaglandin F$_{2\alpha}$ (Ono Pharmaceutical Co., Ltd, Osaka, Japan). The other chemicals used were of analytical grade. All drugs were dissolved in distilled water.

Results

Relaxant effects of (±)-terbutaline and (±)-fenoterol

(±)-Terbutaline and (±)-fenoterol induced concentration-dependent relaxations of the gastric fundus precontracted with PGF$_{2\alpha}$ (Fig. 2). These agonists were full agonists in this preparation producing the same maximum response as (−)-isoprenaline. The pD$_2$ values of (±)-terbutaline and (±)-fenoterol were 4.45 ± 0.10 (n=11) and 5.90 ± 0.09 (n=15) respectively, while intrinsic activities were 1.00 ± 0.03 and 0.99 ± 0.01 respectively. Pretreatment with the selective β$_1$-adrenoceptor antagonist, (±)-atenolol (100 µM), and the selective β$_2$-adrenoceptor antagonist, (±)-butoxamine (100 µM), shifted the concentration-response curves for (±)-terbutaline and (±)-fenoterol to the right without reducing the maximum response (p>0.05) (Fig. 2). In the presence of (±)-atenolol plus (±)-butoxamine, the pD$_2$ values for (±)-terbutaline and (±)-fenoterol were 4.13 ± 0.08 (n=15) and 5.09 ± 0.10 (n=10) respectively, while the intrinsic activities were 0.93 ± 0.03 and 0.93 ± 0.02 respectively. Under conditions where β$_1$- and β$_2$-adrenoceptors were blocked, the potency (pD$_2$ value) of (±)-fenoterol is significantly (p<0.05) higher than that of (±)-terbutaline on the guinea pig gastric fundus.
Effect of (±)-bupranolol on the relaxation to (±)-terbutaline and (±)-fenoterol

In the presence of (±)-atenolol (100 µM) and (±)-butoxamine (100 µM) to block β₁ and β₂ adrenoceptors, the non-selective β₁, β₂ and β₃-adrenoceptor antagonist (±)-bupranolol (3 and 10 µM) produced concentration-dependent rightward shifts of concentration-response curves for (±)-terbutaline and (±)-fenoterol (Fig. 3). The Schild plot of the data revealed the pA₂ values for (±)-bupranolol against (±)-terbutaline and (±)-fenoterol to be 6.21 ± 0.07 and 6.37 ± 0.06 respectively. The slope of each regression line was not significantly different from unity (p>0.05) (Fig. 3). Since higher concentrations of (±)-terbutaline and (±)-fenoterol were needed, we could not use (±)-bupranolol (30 µM) in this study.
Discussion

In the present study, the structure-activity relationships of (±)-terbutaline and (±)-fenoterol were examined on β3-adrenoceptor agonistic activity in the guinea pig gastric fundus. It is generally accepted that (±)-terbutaline and (±)-fenoterol are potent and selective β2-adrenoceptor agonists. The pD₂ values for (±)-terbutaline and (±)-fenoterol reported in the literature are 6.63 and 7.74 respectively for the guinea pig trachea contracted by 1 µM carbachol (Kuällström et al., 1994). In the precontracted gastric fundus, (±)-terbutaline and (±)-fenoterol
induced concentration-dependent relaxations with pD₂ values of 4.45 ± 0.10 and 5.90 ± 0.09 respectively. Furthermore, relaxant responses to (±)-terbutaline and (±)-fenoterol were resistant to blockade by a combination of (±)-atenolol (100 μM) and (±)-butoxamine (100 μM) which normally blocks responses in preparation known to contain β₁ and β₂-adrenoceptors. Therefore, these results suggest that the relaxations in response to (±)-terbutaline and (±)-fenoterol are mainly mediated via β₃-adrenoceptors in the guinea pig gastric fundus.

To confirm the interaction of these agonists with β₃-adrenoceptors, we used the non-selective β₁, β₂ and β₃-adrenoceptor antagonist (±)-bupranolol under conditions designed to assess only β₃-adrenoceptors (Horinouchi and Koike, 1999). In the presence of both (±)-atenolol and (±)-butoxamine, relaxant responses to (±)-terbutaline and (±)-fenoterol were antagonized by (±)-bupranolol. (±)-Bupranolol, at a concentration much higher than that which would antagonize β₁- and β₂-adrenoceptor-mediated effects, antagonized atypical β/β₃-adrenoceptors (Koike et al., 1995; Kaumann and Molenaar, 1996; Malinowska and Schlicker, 1997; Horinouchi and Koike, 1999). These results suggest that (±)-terbutaline- and (±)-fenoterol-induced relaxations were solely mediated by β₃-adrenoceptors in the guinea pig gastric fundus when classical β-adrenoceptors are blocked.

The pA₂ values for (±)-bupranolol obtained in this study resemble those (approximately 6) against catecholamines reported in a previous study on the guinea pig gastric fundus (Horinouchi and Koike, 1999), whereas these values are larger than the pA₂ value (5.29) against (±)-carteolol (Horinouchi and Koike, 2000). (±)-Terbutaline, (±)-fenoterol and catecholamines are arylethanolamines, while (±)-carteolol is an aryloxypropanolamine. It is possible that the β₃-adrenoceptor antagonistic activities of (±)-bupranolol against arylethanolamine are more potent than those against aryloxypropanolamines.

(±)-Terbutaline and (±)-fenoterol have a tert-butyl and aryliso-propyl substituent respectively at the α position on the nitrogen of the ethanolamine side chain. Thus (±)-fenoterol has a structure close to that of (±)-terbutaline, except that the tert-butyl substituent is substituted by the aryliso-propyl substituent, which is a bulky group and may increase the steric bulk and lipophilicity at the end of the alkylamine chain. (±)-Fenoterol was 10-fold more potent than (±)-terbutaline, indicating that an increase in the size of the end of the side chain increases β₃-adrenoceptor agonistic activity in the guinea pig gastric fundus. These results support our previous report concerning atypical β-adrenoceptors of the guinea pig duodenum (Horinouchi and Koike, 2001c).

In summary, the relaxant responses to (±)-terbutaline and (±)-fenoterol, which are resistant to classical β-adrenoceptor antagonists, are predominantly mediated by β₃-adrenoceptors. The structure-activity relationship study of (±)-terbutaline and (±)-fenoterol indicates that arylethanolamines, which have a bulky group on the end of the alkylamine chain, exhibit high potency for β₃-adrenoceptors.

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