The β_2 - and β_3 -Adrenoceptor-Mediated Relaxation Induced by Fenoterol in Guinea Pig Taenia Caecum

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Abstract

Fenoterol, a β_2 -adrenoceptor selective agonist, belongs to the aryethanolamine class. To understand the receptor subtypes responsible for β -adrenoceptor-mediated relaxation of guinea pig taenia caecum, we investigated the effect of fenoterol. Fenoterol caused concentration-dependent relaxation of the guinea pig taenia caecum. Propranolol, bupranolol and butoxamine produced shifts of the concentration-response curve for fenoterol. Schild regression analyses carried out for propranolol, butoxamine and bupranolol against fenoterol gave pA_2 values of 8.41, 6.33 and 8.44, respectively. However, in the presence of 3×10^{-4} M atenolol, 10^{-4} M butoxamine and 10^{-6} M phentolamine to block the β_1 -, β_2 - and α -adrenoceptor effects, respectively, Schild regression analysis carried out for bupranolol against fenoterol gave pA_2 values of 5.80. These results suggest that the relaxant response to fenoterol in the guinea pig taenia caecum is mediated by both the β_2 - and the β_3 -adrenoceptors.

Key words: β_2 -adrenoceptor, β_3 -adrenoceptor, fenoterol, guinea pig taenia caecum

Introduction

The β -adrenoceptors belong to a large family of G-protein coupled receptors that are characterized by seven transmembrane helices. Three subtypes of β -adrenoceptors (β_1 , β_2 and β_3) have been characterized by pharmacological, biochemical and molecular biological cloning approaches, and at present for each subtype (β_1 , β_2 and β_3), the structure has been identified in turn (Strosberg, 1997). A large body of evidence indicates that the β_3 -adrenoceptors occur in the intestinal smooth muscle of different species, including humans, with a function of inhibiting muscle contractility (Bianchetti and Manara, 1990; De Ponti *et al.*, 1996).

We had demonstrated that the 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; 1995b), although the β_1 -adrenoceptors are not involved (Koike *et al.*, 1994). Our previous studies also showed that the relaxant responses to isoprenaline and salbutamol, which belong to the

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arylethanolamine class, are mediated by both the β_2 - and the β_3 -adrenoceptors in the guinea pig taenia caecum (Koike *et al.*, 1997). Fenoterol, a β_2 -adrenoceptor selective agonist, has arylisopropyl substituent at the α position on the nitrogen of the ethanolamine side chain. This drug has an asymmetric carbon, and a mixture of equal part of its stereoisomer is used for the treatment of bronchial asthma and in animal experiments. Moreover, fenoterol bears a structural resemblance to isoprenaline or salbutamol (Horinouchi *et al.*, 2001; Horinouchi and Koike, 2001). However, it is not known whether the β_3 -subtype is involved in the β -adrenoceptor-mediated relaxation induced by fenoterol. Therefore, we have studied in detail the β -adrenoceptor-mediated relaxation of guinea pig taenia caecum by measuring the potencies of fenoterol.

Materials and Methods

Mechanical responses

Male guinea pigs weighing 300–500 g were killed by cervical dislocation and a 2 to 3-cm piece of the taenia caecum was isolated and suspended in a 20-ml organ bath filled with a Ringer-Locke solution (NaCl, 154; KCl, 5.6; CaCl₂, 2.2; MgCl₂, 2.1; NaHCO₃, 5.9 and glucose, 2.8 mM) kept at 32°C and bubbled with a mixture of 95% O₂ and 5% CO₂. The mechanical responses of the smooth muscle preparations were recorded isotonically under a tension of 0.7 g. The experiments were started after the preparations had been allowed to develop their spontaneous tone for 2 h. The concentration-response curves for the agonists were obtained cumulatively and the relaxation induced by these drugs was expressed as a percentage of the maximal relaxation produced by 3×10^{-7} M isoprenaline, the reference drug. To test the antagonism, 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 60 min. The spontaneous smooth muscle tone was reproducible when taenia caecum pieces were without the load. In our previous experiments, after the control concentration-response curves were determined, two or three successive cumulative concentration-response curves for isoprenaline were determined. The curves were nearly superimposable and changes in sensitivity (sensitization or desensitization) were slight (data not shown). Six or more concentration-response curves could be made in succession. In some experiments, atenolol (3×10^{-4} M), butoxamine (10^{-4} M) and phentolamine (10^{-6} M) were added to inhibit the β_1 -, β_2 - and α -adrenoceptors, respectively. Agonistic potency was expressed as the pD₂ value (Van Rossum, 1963). The competitive antagonistic potency was expressed as the pA₂ value. It was calculated according to the method of Tallarida *et al.* (1979), which was originally described by Arunlakshana and Schild (1959).

Data analysis

Numerical results are expressed as means $\pm$ S.E.M. and statistical analyses were performed with the Newman-Keuls test when appropriate. A P value of less than 0.05 was considered significant.

Fig. 1. Antagonism of fenoterol-induced relaxation by propranolol. Control (●), propranolol 10^{-8} M (○), 3×10^{-8} M (△), 10^{-7} M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of fenoterol. Each point represents the mean $\pm$ S.E.M. of six experiments.

Drugs

The drugs used were obtained from the following sources: isoprenaline hydrochloride, fenoterol hydrochloride, butoxamine hydrochloride, propranolol hydrochloride (Sigma-Aldrich Co., St. Louis, MO, U.S.A.); bupranolol hydrochloride (Looser; Kaken Seiyaku Co., Tokyo, Japan); atenolol (Research Biochemicals, Natick, MA, U.S.A.); and phentolamine mesylate (Ciba Geigy, Basel, Switzerland). All the drugs were dissolved in distilled water. The other chemicals used were of analytical grade.

Results

Fenoterol caused graded relaxation of the guinea pig taenia caecum piece in which the tone had been raised spontaneously, with the pD_2 value of 7.60 ± 0.08 (Fig. 1). Propranolol (10^{-8} – 10^{-7} M) competitively antagonized the relaxant responses to fenoterol (Fig. 1). The Schild plot of the data gave the pA_2 value of 8.41 ± 0.06 and the slope of the regression line (1.10 ± 0.08) was not significantly different from unity. Butoxamine (10^{-6} – 10^{-5} M), a β_2 -selective antagonist, caused competitive antagonism of the relaxant responses (Fig. 2). The Schild plot of the data gave the pA_2 value of 6.33 ± 0.02 and the slope of the regression line (1.06 ± 0.04) was not significantly different from unity.

Bupranolol (10^{-8} – 10^{-7} M) caused competitive antagonism of the relaxant responses (Fig. 3). The Schild plot of the data gave the pA_2 value of 8.44 ± 0.04 and the slope of the regression line (1.05 ± 0.04) was not significantly different from unity. In the presence of 3×10^{-4} M atenolol, 10^{-4} M butoxamine and 10^{-6} M phentolamine to block the β_1 -, β_2 - and α -adrenoceptor effects, respectively, bupranolol (3×10^{-6} – 10^{-5} M) also caused competitive antagonism of the relaxant responses (Fig. 4). However, the Schild plot of the data gave the pA_2 value of 5.80 ± 0.03 and the slope of the regression line (1.04 ± 0.04) was not significantly different from unity. The

Fig. 2. Antagonism of fenoterol-induced relaxation by butoxamine. Control (●), butoxamine 10^{-6} M (○), 3×10^{-6} M (△), 10^{-5} M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of fenoterol. Each point represents the mean $\pm$ S.E.M. of six experiments.

Fig. 3. Antagonism of fenoterol-induced relaxation by bupranolol. Control (●), bupranolol 10^{-6} M (○), 3×10^{-8} M (△), 10^{-7} M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of fenoterol. Each point represents the mean $\pm$ S.E.M. of six experiments.

difference between the pA_2 values for bupranolol in the presence and in the absence of the β_1 , β_2 - and α -blockers was statistically significant ($P < 0.05$).

Discussion

Previously, we have demonstrated that both the β_2 - and the β_3 -adrenoceptors are involved in the β -adrenoceptor-mediated relaxation of the guinea pig taenia caecum (Koike *et al.*, 1994; 1995a; 1995b). In the present studies, fenoterol-induced relaxation of the guinea pig taenia caecum was antagonized in a concentration-dependent manner by butoxamine, a β_2 -selective

Fig. 4. Antagonism of fenoterol-induced relaxation by bupranolol in the presence of 3×10^{-4} M atenolol, 10^{-4} M butoxamine and 10^{-6} M phentolamine. Fenoterol in the absence of blockers (●), fenoterol in the presence of blockers (■), with bupranolol 3×10^{-6} M (○), or 10^{-5} M (△). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of fenoterol. Each point represents the mean $\pm$ S.E.M. of six experiments.

antagonist. The Schild slope of 1.06 indicates the competitive nature of the antagonism. The pA_2 value for butoxamine calculated from the Schild plot (6.33) is in good agreement with the generally accepted value (6.20) (Koike *et al.*, 1994), indicating a predominance of β_2 -adrenoceptors.

Propranolol and bupranolol antagonized the response to fenoterol in a concentration-dependent manner. Schild slopes of 1.10 and 1.05 observed with propranolol and bupranolol are consistent with the competitive nature of the antagonism. The pA_2 values for propranolol and bupranolol are in good agreement with the generally accepted value. Moreover, in the presence of 3×10^{-4} M atenolol, 10^{-4} M butoxamine and 10^{-6} M phentolamine to block the β_1 -, β_2 - and α -adrenoceptor effects, respectively, bupranolol also shifted to the right the concentration-response curves for fenoterol. Schild slope of 1.04 observed with bupranolol in the presence of blockers indicates the competitive nature of the antagonism. However, a Schild regression analysis carried out for bupranolol against fenoterol gave pA_2 value of 5.80, which was significantly different from the value (8.44) obtained in the absence of the blockers. Bupranolol was used in our study since it acts as an antagonist at the β_3 -adrenoceptors (Arch and Kaumann, 1993; Blin *et al.*, 1993), although at concentrations much higher than those necessary for the blockade of the β_1 - or the β_2 -adrenoceptors (Kaumann, 1996; Kaumann and Molenaar, 1996; Malinowska and Schlicker, 1996). It has been suggested that, at low concentrations (nM), bupranolol is a non-selective β_1 - and β_2 -adrenoceptor antagonist and that at high concentrations (mM), bupranolol is a selective β_3 -adrenoceptor antagonist (Kaumann, 1989). These results suggest that the relaxant response of the guinea pig taenia caecum to the high concentration of fenoterol may be mediated by the β_3 -adrenoceptors in the presence of β_1 -, β_2 - and α -blockers. The present results are in good agreement with our previous report (Koike *et al.*, 1997), which showed that the relaxant responses to isoprenaline and salbutamol are mediated by both the β_2 -

and the β_3 -adrenoceptors. Therefore, the present findings indicate that fenoterol produce concentration-dependent relaxation in this smooth muscle preparation through action of both the β_2 - and the β_3 -adrenoceptors. Moreover, the activity of fenoterol in the absence of blockers was about 200-fold more potent than that in the presence of blockers. Those findings prove that the low concentration of fenoterol acts as a β_2 -adrenoceptor selective agonist and that the high concentration of fenoterol acts as an agonist at the β_3 -adrenoceptors.

In conclusion, the main objective of the present studies was to clarify whether β_3 -subtype is involved in the β -adrenoceptor-mediated relaxation induced by fenoterol in the guinea pig taenia caecum. Our results suggest that the relaxant response to fenoterol in the guinea pig taenia caecum is mediated by both the β_2 - and the β_3 -adrenoceptors.

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