Adrenaline produces the relaxation of guinea-pig airway smooth muscle primarily through the mediation of $\beta_2$-adrenoceptors

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Abstract

The $\beta$-adrenoceptor subtype that mediates adrenaline-induced relaxation was pharmacologically identified in smooth muscle cells of the isolated guinea-pig trachea. Adrenaline produced a concentration-dependent relaxation with a $pD_2$ value of 7.1. The concentration-response curve for adrenaline was shifted rightwards in a competitive fashion by the $\beta_1$-/ $\beta_2$-nonselective antagonists propranolol and bupranolol, with $pA_2$ values of 8.85 and 8.97, respectively. Adrenaline-induced relaxation was not affected by the $\beta_1$-selective antagonists atenolol and CGP-20,712A within the concentration ranges supposed to antagonize the $\beta_1$-subtype (atenolol, $\leq 10^{-6}$ M; CGP-20,712A, $\leq 10^{-8}$ M). By contrast, the concentration-response curve for adrenaline was shifted rightwards in a competitive fashion by atenolol at concentrations $\geq 3 \times 10^{-6}$ M with a $pA_2$ value of 5.77. The concentration-response curve for adrenaline was also competitively antagonized by the $\beta_2$-selective antagonists butoxamine and ICI-118,551 with $pA_2$ values of 6.86 and 8.73, respectively. The $pA_2$ values of $\beta$-adrenoceptor antagonists (propranolol, bupranolol, atenolol, butoxamine and ICI-118,551) tested against adrenaline were consistent with the values when tested against salbutamol, a $\beta_2$-selective adrenoceptor agonist. The present findings provide evidence that the relaxant response of the smooth muscle of the guinea-pig trachea to the adrenal medulla hormone, adrenaline, is mainly mediated through $\beta_2$-adrenoceptors.

Key words: adrenaline, $\beta_2$-adrenoceptor, butoxamine, ICI-118,551, relaxation, salbutamol, tracheal smooth muscle

Introduction

The $\beta$-adrenoceptor is a member of the G-protein coupled receptor (GPCR) family and is now classified into three subtypes ($\beta_1$, $\beta_2$ and $\beta_3$) (Arch and Kaumann, 1993; Johnson, 1998; Nagatomo and Koike, 2000). Of these subtypes, the $\beta_2$-adrenoceptor plays the most important...
role in the relaxation of airway smooth muscle (Johnson, 1998; Lands et al., 1967; Nagatomo and Koike, 2000; Torphy, 1994). This view is supported by the following evidence: 1) the three catecholamines that produce airway smooth muscle relaxation are ranked in potency as isoprenaline > adrenaline > noradrenaline (Lands et al., 1967; Nagatomo and Koike, 2000; Tanaka et al., 2003); 2) β2-receptors are richly expressed in airway smooth muscle (Barnes, 1993; Johnson, 1998; Nagatomo and Koike, 2000; Nijkamp, 1993); 3) β2-selective adrenoceptor agonists, widely used for the treatment of asthma, strongly relax this smooth muscle (Brittain et al., 1968; Waldeck, 2002). Therefore, it is generally believed that the catecholamine-induced relaxation of airway smooth muscle is mainly mediated via the β2-subtype of adrenoceptor (β2-receptor). Indeed, based on the pharmacological evidence obtained with multiple β1- and β2-selective antagonists with different pA2 values, we have shown that the primary β-adrenoceptor that mediates the relaxant response to isoprenaline, a synthetic catecholamine that nonselectively recognizes the three β-adrenoceptor subtypes (Johnson et al., 1993), is the β2- but not the β1-receptor in guinea-pig tracheal smooth muscle (Tanaka et al., 2004b). This result further suggests an important role for the β2-receptor as the key β-adrenoceptor that controls airway smooth muscle tone and for it being the targeted membrane protein for the pharmacologic therapy of asthma.

On the other hand, there are few studies on the β-adrenoceptor subtype that mediates the relaxation of airway smooth muscle in response to endogenous catecholamines including adrenaline (Lemoine et al., 1985; Lemonine et al., 1989; O’Donnell and Wanstall, 1981). While these earlier studies have suggested that the β-adrenoceptor subtype mediating adrenaline-induced relaxation is principally β2 rather than β1 (Lemoine et al., 1985; Lemonine et al., 1989), this conclusion is based mainly on an analysis using ICI-118,551 (Bilski et al., 1983) as the exclusive β-adrenoceptor antagonist. Furthermore, it is not certain whether the inhibition of the relaxant effect of adrenaline by ICI-118,551 occurs in a competitive manner, since the slope of the Schild plot analysis against adrenaline was less than unity (O’Donnell and Wanstall, 1981). In the present study, we provide pharmacological evidence to show that the β2-receptor is the primary subtype involved in the mediation of the relaxant response of guinea-pig tracheal smooth muscle to adrenaline, an adrenal medulla hormone, as is also the case for responses to salbutamol, the well known β2-selective adrenoceptor agonist (Brittain et al., 1968; Koike et al., 2004).

**Materials and methods**

Male and female Hartley guinea-pigs weighing 300–600 g (Saitama Experimental Animals, Saitama, Japan) were used in the present study. Guinea-pigs were housed under standard laboratory conditions on a 12-h light/dark cycle (lights on 8 AM; lights off 8 PM) in rooms in which the temperature (20–22°C) and the relative air humidity (50 ± 5%) were strictly regulated. Food and water were available ad libitum to all animals. The study was conducted in accordance with the Guideline for the Care and Use of Laboratory Animals adopted by the Committee on the Care and Use of Laboratory Animals of Toho University School of Pharmaceutical Sciences (accredited by The Ministry of Education, Culture, Sports, Science, and Technology, Japan).
Preparation of tracheal tissue

Guinea-pigs were killed by cervical dislocation and exsanguinated from the common carotid or external iliac artery. The trachea was carefully isolated and immersed in Ringer-Locke solution (mM: NaCl, 154.0; KCl, 5.6; CaCl$_2$, 2.2; MgCl$_2$, 2.1; NaHCO$_3$, 5.9; glucose, 2.8) bubbled with a mixture of 95% O$_2$ and 5% CO$_2$. The tracheal preparations were very cautiously cleaned of unnecessary adipose and connective tissue under a dissecting microscope to ensure that the smooth muscle was not damaged. Subsequently, the tracheal cartilage containing the smooth muscle was cut into ring preparations of 2-mm in length. The intimal surface of the tracheal tissue was gently rubbed with moistened filter paper to remove tracheal epithelium as much as possible.

Measurement of tension changes

Preparations were suspended using stainless steel hooks (outer diameter, 200 µm) in a 5-ml organ bath (UC-5; UFER Medical Instrument, Kyoto, Japan) containing Ringer-Locke solution which was maintained at $32 \pm 1 ^\circ C$ and bubbled with the O$_2$-CO$_2$ mixture. Tension changes were isometrically recorded with a force-displacement transducer (T7-8-240; Orientec, Tokyo, Japan) connected to an amplifier (high-gain DC amplifier: model, AD 632J; Nihon Kohden, Tokyo, Japan).

Assessment of the relaxant effects of adrenaline

The relaxant effects of adrenaline were examined as follows. After stretching the preparation with an initial load of 2 g, a spontaneous tension development was generated. During this tension development, the bath solution was renewed every 20 min for 60 min. This spontaneous tension development lasted for several hours without appreciable decline. Following the 60 min tension development period, the muscle was contracted with histamine ($10^{-5}$ M) for 15 min, and this was then washed out. After this procedure, the tracheal preparations were incubated for a further 60 min with renewal of the bath solution every 20 min, and were then again contracted with $10^{-5}$ M histamine. When the active tension obtained with histamine and the initial muscle stretch reached a steady-state level about 30 min after the application of histamine, adrenaline was cumulatively applied to the bath medium until the maximum relaxant responses to adrenaline were obtained. At the end of each experiment, papaverine ($10^{-4}$ M) was applied to the bath medium to obtain the substantially maximum relaxant response.

Assessment of the effects of β-adrenoceptor antagonists

Antagonistic effects of test drugs (propranolol, bupranolol, atenolol, CGP-20,712A, butoxamine, ICI-118,551) were examined as follows. One of the β-adrenoceptor antagonists was applied to the bath medium simultaneously with histamine ($10^{-5}$ M). Thirty minutes after the addition of the β-adrenoceptor antagonist, a concentration-response curve for adrenaline was constructed in both the absence and presence of the β-adrenoceptor antagonist. In this study, only a single concentration-response curve was obtained per preparation, whether in the absence or in the presence of β-adrenoceptor antagonists, to prevent possible tachyphylaxis,
unexpected interventions by β-adrenoceptor antagonists, or abnormal relaxant responses due to muscle fatigue after repetitive relaxations in the presence of varied concentrations of β-adrenoceptor antagonists.

**Drugs**

(±)-Bupranolol hydrochloride was kindly donated by Kaken Pharmaceutical Co., Ltd. (Tokyo, Japan). Other drugs used in the present study were as follows: histamine dihydrochloride, (+)-adrenaline (+)-bitartrate, salbutamol (albuterol; α-[t-butylamino)methyl]-4-hydroxy-m-xylene-α,α'-diol) hemisulfate, (±)-propranolol hydrochloride, (±)-butoxamine hydrochloride, CGP-20,712A ((±)-2-hydroxy-5-[2-[2-hydroxy-3-[1-methyl-4-(trifluoromethyl)-1H-imidazol-2-yl]phenoxy]propyl]amino|ethoxy]-benzamide methanesulfonate), papaverine hydrochloride (Sigma-Aldrich, St. Louis, Mo., USA); ICI-118,551 ((±)-1-[2,3-(dihydro-7-methyl-1H-inden-4-yl)oxy]-3-(1-methylethyl)amino]-2-butanol hydrochloride), (±)-atenolol (Sigma-RBI, Natick, Mass., USA). All other chemicals used were of analytical grade. Distilled water was used to dissolve and dilute all drugs.

**Data analysis and statistics**

To construct concentration-response relationships for adrenaline and salbutamol, the percentages of the relaxant responses were calculated considering the tension level before application of adrenaline or salbutamol (spontaneous tone plus histamine-elicited tension) to be 0% and the maximum relaxation obtained after application of papaverine (10⁻⁴ M) to be 100%. Data were plotted as a function of adrenaline or salbutamol concentration and fitted to the equation:

\[
E = E_{\text{max}} \times \frac{A^n}{(EC_{50}^n + A^n)}
\]

where \(E\) is the % relaxation at a given relaxant concentration of either adrenaline or salbutamol, \(E_{\text{max}}\) is the maximum relaxation, \(A\) is the concentration of relaxants, \(n\) is the slope function and \(EC_{50}\) is the relaxant concentration that produced a 50% response. The curve fitting was carried out using GraphPad Prism™ (Version 3.00) (GraphPad Software, San Diego, Calif., USA). The \(EC_{50}\) values were converted to logarithmic values (\(\text{pD}_2\), -log\(EC_{50}\)) for statistical analysis. The competitive antagonistic potency was expressed as a \(pA_2\) value, which was calculated according to the method originally reported by Arunlakshana and Schild (1959).

Data are presented as the mean ± SEM or as the mean value with 95% confidence intervals in parentheses, and \(n\) refers to the number of experiments. The significance of the difference between mean values was evaluated with GraphPad Prism™ using the unpaired Student’s \(t\)-test, or the unpaired \(t\)-test with Welch’s correction if necessary. A \(P\) value of less than 0.05 was considered to be statistically significant.

**Results**

**Effects of propranolol and bupranolol on the relaxation to adrenaline**

In the isolated guinea-pig tracheal smooth muscle, adrenaline (Figs 1A · C, 2A, 3A · D: open circles) as well as salbutamol (Figs 2B, 3B · E: open circles) elicited a full relaxation in a
Adrenaline-induced tracheal relaxation

Concentration-dependent manner. \( pD_2 \) Values of adrenaline and salbutamol were 7.11 ± 0.05 (n=27) and 7.67 ± 0.05 (n=14), respectively.

Figure 1 shows the effects of both propranolol and bupranolol, which antagonize \( \beta_1 \)-/\( \beta_2 \)-adrenoceptors nonselectively, on the relaxation in response to adrenaline. The concentration-response curve for adrenaline was shifted rightwards in a parallel fashion by both propranolol and bupranolol. The regression lines of the Schild plot for propranolol (Fig. 1B) and bupranolol (Fig. 1D) were not significantly different from unity, which indicates a competitive antagonism by both antagonists of the relaxant response to adrenaline (Table 1). The \( pA_2 \) values for propranolol and bupranolol against adrenaline were calculated as 8.85 and 8.97, respectively, which were consistent with the values against salbutamol (8.69 and 8.94) (Table 1).

**Effects of atenolol and CGP-20,712A on the relaxation to adrenaline**

The concentration-response curve for adrenaline was not affected by atenolol within the concentration ranges \( \leq 10^{-6} \text{ M} \) (n=3). The relaxant response to adrenaline was also not affected by CGP-20,712A (\( \leq 10^{-8} \text{ M} \)) (n=3). However, the concentration-response curve for adrenaline (Fig. 2A) as well as that for salbutamol (Fig. 2B) was shifted rightwards in a parallel manner by higher concentrations (3 \( \times 10^{-6} - 3 \times 10^{-5} \text{ M} \)) of atenolol. Schild plot analysis carried out for atenolol against adrenaline and salbutamol yielded straight lines with slopes of unity (Fig. 2C; Table 1), providing \( pA_2 \) values of 5.77 against adrenaline and 5.73 against salbutamol (Table 1).
Figure 3A and Fig. 3B show the effects of butoxamine on the relaxations to both adrenaline and salbutamol. Relaxations to both adrenaline and salbutamol were competitively antagonized by butoxamine, which was evidenced by the Schild plot analysis providing straight lines of slopes of unity (Fig. 3C; Table 1). The pA₂ values of butoxamine against adrenaline and salbutamol were identical at 6.86 and 6.85 respectively (Table 1). Similarly, relaxations to both adrenaline (Fig. 3D) and salbutamol (Fig. 3E) were competitively antagonized by ICI-118,551. Its pA₂ values against adrenaline (8.73) and salbutamol (8.89) were also not significantly different (P>0.05) from each other (Fig. 3F, Table 1).

Table 1  Schild plot analysis for β-adrenoceptor antagonists against adrenaline- and salbutamol-elicited relaxations of guinea-pig tracheal smooth muscle

| Subtype selectivity | Antagonists  | Adrenaline | Salbutamol |
|---------------------|-------------|------------|------------|
|                     | pA₂ (95% C.I.) | n | pA₂ (95% C.I.) | n |
| β₁/β₂               | (±)-Propranolol | 8.85 (8.60–9.24) (15) | 8.69 (8.42–9.12) (12) |
|                     | (±)-Bupranolol | 8.97 (8.80–9.19) (21) | 8.94 (8.73–9.28) (9) |
| β₁                  | (±)-Atenolol  | 5.77 (5.58–6.08) (18) | 5.73 (5.63–5.88) (18) |
|                     | [0.91 (0.67–1.16)] | | [1.02 (0.87–1.18)] |
| β₂                  | (±)-Butoxamine | 6.86 (6.63–7.27) (12) | 6.85 (6.69–7.08) (11) |
|                     | [0.93 (0.64–1.22)] | | [1.04 (0.82–1.26)] |
|                     | ICI-118,551   | 8.73 (8.34–9.74) (11) | 8.89 (8.60–9.36) (11) |
|                     | [0.94 (0.52–1.36)] | | [1.09 (0.81–1.37)] |

Each value represents mean values with 95% confidence intervals (C.I.). Values from Tanaka et al., 2004b.

Effects of butoxamine and ICI-118,551 on the relaxations to adrenaline and salbutamol

Figure 3A and Fig. 3B show the effects of butoxamine on the relaxations to both adrenaline and salbutamol. Relaxations to both adrenaline (Fig. 3A) and salbutamol (Fig. 3B) were competitively antagonized by butoxamine, which was evidenced by the Schild plot analysis providing straight lines of slopes of unity (Fig. 3C; Table 1). The pA₂ values of butoxamine against adrenaline and salbutamol were identical at 6.86 and 6.85 respectively (Table 1). Similarly, relaxations to both adrenaline (Fig. 3D) and salbutamol (Fig. 3E) were competitively antagonized by ICI-118,551. Its pA₂ values against adrenaline (8.73) and salbutamol (8.89) were also not significantly different (P>0.05) from each other (Fig. 3F, Table 1).
Discussion

In this study, using several types of $\beta$-adrenoceptor antagonists with different $pA_2$ values, we reached the conclusion that adrenaline as well as salbutamol produces relaxation of guinea-pig tracheal smooth muscle through the mediation of $\beta_2$-receptors. This deduction was based on the following observations.

Firstly, we showed that adrenaline-elicited tracheal relaxation is competitively antagonized by propranolol and bupranolol, both of which nonselectively antagonize $\beta_1$-/$\beta_2$-subtypes. This finding indicates that the adrenaline-elicited relaxation of tracheal muscle is mediated through typical $\beta$-adrenoceptors, though this finding does not directly indicate the involvement of $\beta_2$-receptors.

Secondly, adrenaline-elicited tracheal relaxation was found not to be affected by atenolol ($\leq 10^{-6}$ M) and CGP-20,712A ($\leq 10^{-4}$ M) within their concentration ranges that are supposed to selectively antagonize the $\beta_1$-subtype: $pA_2$ values of atenolol and CGP-20,712A against the $\beta_1$-subtype, when obtained in isoprenaline-elicited relaxation in the guinea-pig esophageal smooth muscle.
muscle, are ≈ 7.0 (7.01) (Tanaka et al., 2004a) and ≈ 9.5 (9.43) (Horinouchi et al., 2003), respectively. On the other hand, ≥ 3 × 10⁻⁶ M atenolol competitively antagonized the relaxant response to adrenaline (Fig. 2A), which was strongly supported by the unitary slope of the Schild plot analysis against adrenaline (Fig. 2C, Table 1). The pA₂ value of atenolol against adrenaline was 5.77 and this was consistent with the value against salbutamol (5.73), which indicates the mediation of β₂-receptor in the response to adrenaline (Table 1). These pA₂ values of atenolol were in good agreement with the previously reported values against fenoterol (5.61) (O'Donnell and Wanstall, 1979); and salbutamol (5.71, 5.76) (Keith et al., 1986; Tanaka et al., 2004b). These observations rule out the β₁ but support the β₂ as the primary β-adrenoceptor subtype that mediates the relaxation of tracheal smooth muscle to adrenaline.

Thirdly, we showed that tracheal relaxation to adrenaline as well as to salbutamol was competitively antagonized by butoxamine (Fig. 3A–C) or ICI-118,551 (Fig. 3D–F). Their pA₂ values against adrenaline (6.86 and 8.73) were consistent with the values against salbutamol (6.85 and 8.89) (Table 1), and thus the mediation of β₂-receptors in the adrenaline-induced response was strongly supported. The pA₂ values of butoxamine and ICI-118,551 were also in agreement with previously reported values against β₂-selective agonists: butoxamine, 6.68 (against salbutamol in guinea-pig taenia caecum) (Koike et al., 1997); ICI-118,551, 8.78 and 9.17 (against salbutamol in guinea-pig trachea) (Keith et al., 1986; O'Donnell and Wanstall, 1981), and 8.81 (against fenoterol in guinea-pig trachea) (O'Donnell and Wanstall, 1981).

In conclusion, we have provided evidence to show that the β₂ rather than the β₁-adrenoceptor plays the primary role in mediating the relaxant response to adrenaline in guinea-pig tracheal smooth muscle. The present study is the first identification of the β-adrenoceptor subtype mediating adrenaline-induced relaxation of tracheal smooth muscle based on a systematic pharmacological approach using multiple, subtype-selective β-adrenoceptor antagonists with different affinities.

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