The $\beta_3$-Adrenoceptor-Mediated Relaxation Induced by Epinephrine in Guinea Pig Taenia Caecum

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Abstract

The mechanisms of the $\beta$-adrenoceptor-mediated relaxation induced by epinephrine in guinea pig taenia caecum were examined. The relaxant response to epinephrine was unaffected by propranolol ($\sim 10^{-4}$ M) or phentolamine ($\sim 10^{-5}$ M). The response to epinephrine was antagonized in a concentration-dependent manner by butapranolol, and Schild plot of the data revealed the $pA_2$ value of 5.87. Epinephrine significantly increased cyclic AMP level in this preparation. Butapranolol ($10^{-4}$ M) significantly decreased the cyclic AMP level that was elicited by epinephrine, whereas propranolol ($10^{-3}$ M) produced no effect. These results suggest that the relaxant response to epinephrine in the guinea pig taenia caecum is mainly mediated by $\beta_3$-adrenoceptors.

Key words: $\beta_3$-adrenoceptor, epinephrine, guinea pig taenia caecum

Introduction

The $\beta$-adrenoceptors were originally subclassified by Lands et al. (1967) in the $\beta_1$- and $\beta_2$-subtypes based on the agonist potency and tissue localization. Recently, existence of the $\beta_3$-adrenoceptor has been revealed in different tissues by binding and functional studies (Arch and Kaumann, 1993). A large body of evidence indicates that the $\beta_3$-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 $\beta_2$- and $\beta_3$-adrenoceptors are involved in the $\beta$-adrenoceptor-mediated relaxation of the guinea pig taenia caecum (Koike et al., 1994; 1995a; 1995b), although the $\beta_2$-adrenoceptors are not involved (Koike et al., 1994). Our previous studies also showed that the relaxant responses to norepinephrine, a neurotransmitter, CGP12177, a non-conventional partial agonist, and BRL37344, a $\beta_3$-adrenoceptor-selective agonist, in the guinea pig taenia caecum are mediated by $\beta_3$-adrenoceptors (Koike et al., 1995b; 1995c; 1997). However, it is not known whether the $\beta_3$-subtype is involved in the $\beta$-adrenoceptor-mediated relaxation induced by epinephrine, another neurotransmitter of sympathetic nervous system.
system. Therefore, the aim of this study is to examine the mode of the action of epinephrine in the guinea pig taenia caecum.

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 agonists 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. 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).

Estimation of cyclic AMP levels

Tissue concentrations of cyclic AMP were estimated by the method of Steiner et al. (1972). Two pieces of taenia were removed from one caecum and suspended in two similar 20 ml baths filled with a Ringer-Locke solution kept at 32°C and gassed with a mixture of 95% O₂ and 5% CO₂. They were frozen in liquid nitrogen immediately after the test drugs were added to the baths for 2 min (Koike and Takayanagi, 1983). One piece was used for measuring the control level of cyclic AMP and the other for estimating any change of cyclic AMP concentration after treatment with the test drugs. The tissues were homogenized with a glass homogenizer in 2 ml of cold trichloroacetic acid (6% w/v). The homogenate was centrifuged at 3,000 rpm at 0°C for 15 min, the supernatant was then acidified with 1 N HCl, and the trichloroacetic acid was extracted with ether. The cyclic AMP samples were lyophilized. The lyophilized samples were dissolved with sodium acetate buffer, pH 6.2, and used for the estimation of cyclic AMP. The quantity of cyclic AMP was determined by a radioimmunoassay, using a commercial kit (Yamasa, Chiba, Japan). The radioimmunoassay for cyclic AMP was carried out in 0.05 M
sodium acetate buffer, pH 6.2. Assays were performed in nonsiliconized disposable culture tubes. Each tube contained 100 ml of cyclic AMP standard or unknown solution, 100 ml of antibody, and 100 ml of the 125I-cyclic AMP in a final volume of 500 ml. The reaction mixture then incubated 18 or 24 hours at 4°C. Activated carbon was used for separating bound and free 125I-ligand before centrifugation at 4°C for 15 min, and the precipitate was counted in a gamma spectrometer (Aloka ARC-370M, Tokyo, Japan).

Protein assay
Protein concentrations were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard.

Data analysis
Numerical results were expressed as means ± S.E. and statistical analyses were performed with the Newman-Keuls test when appropriate. A P value of less than 0.05 was considered significant.

Drugs
The drugs used were obtained from the following sources: isoprenaline hydrochloride, epinephrine bitartrate, propranolol hydrochloride, butoxamine hydrochloride (Sigma Chemical Co., St. Louis, MO, U.S.A.); bupranolol hydrochloride (Looser; Kaken Seiyaku Co., Tokyo, Japan); atenolol (Research Biochemicals, Natick, MA, USA); prazosin hydrochloride, yohimbine hydrochloride (Wako Pure Chemicals Industries, Osaka, Japan) and phentolamine mesylate (Ciba Geigy, Basel, Switzerland). All the drugs were dissolved in distilled water. The other chemicals used were of analytical grade.

Results

Mechanical responses
Epinephrine caused graded relaxation of the guinea pig taenia caeca piece in which the tone had been raised spontaneously, with the pD2 value of 7.39 ± 0.06 (Fig. 1). Propranolol (~10⁻⁵ M), a nonselective β₁- and β₂-adrenoceptor antagonist, did not significantly affect the relaxant response to epinephrine (Fig. 1). Moreover, phenolamine (~10⁻⁵ M), a nonselective α₁- and α₂-adrenoceptor antagonist, did not significantly affect the relaxant response to epinephrine (Fig. 2). Atenolol (~3 × 10⁻⁴ M, a selective β₁-adrenoceptor antagonist), butoxamine (~10⁻⁴ M, a selective β₂-adrenoceptor antagonist), prazosin (~10⁻⁵ M, a selective α₁-adrenoceptor antagonist) and yohimbine (~10⁻⁵ M, a selective α₂-adrenoceptor antagonist) had no effect on the potency of epinephrine (data not shown).

Bupranolol (3 × 10⁻⁴-3 × 10⁻³ M) caused competitive antagonism of the relaxant responses (Fig. 3). The Schid plot of the data gave the pA₂ value of 5.87 ± 0.04 and the slope of the regression line (1.10 ± 0.05) was not significantly different from unity. Bupranolol itself had no effect on the degree of tone.
Fig. 1. Effect of propranolol on the concentration-response curve for epinephrine in the guinea pig taenia caecum. Control (●), propranolol $10^{-8}$ M (○), $3 \times 10^{-8}$ M (△), $10^{-7}$ M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline ($3 \times 10^{-7}$ M), and abscissa: log concentration (M) of epinephrine. Each point represents the mean ± S.E. of six experiments.

Fig. 2. Effect of phenolamine on the concentration-response curve for epinephrine in the guinea pig taenia caecum. Control (●), phenolamine $10^{-8}$ M (○), $3 \times 10^{-8}$ M (△), $10^{-7}$ M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: log concentration (M) of isoprenaline. Each point represents the mean ± S.E. of six experiments.
Cyclic AMP levels

The maximal concentration (3 × 10^{-7} M) of isoprenaline increased cyclic AMP level in the guinea pig taenia caecum (basal level, 3.31 ± 0.05 pmol/mg protein; with isoprenaline, 5.21 ± 0.28 pmol/mg protein). When the guinea pig taenia caecum was incubated with epinephrine (3 × 10^{-4} M) for 2 min, cyclic AMP level (5.14 ± 0.16 pmol/mg protein) was increased by 1.55-fold (P < 0.05) over that of the basal level (Fig. 4). When taenia caecum was incubated with bupranolol (10^{-4} M) in combination with epinephrine, the cyclic AMP level (3.57 ± 0.13 pmol/mg protein) was significantly decreased (P < 0.05) (Fig. 4). However, propranolol (10^{-3} M) produced no effect (5.08 ± 0.26 pmol/mg protein) (Fig. 4). Bupranolol and propranolol had no effect on the basal cyclic AMP level (data not shown).
Discussion

Previous study has suggested that the relaxant response to norepinephrine in the guinea pig taenia caecum is mainly mediated by \( \beta \)-adrenoceptor-induced stimulation and activation of the adenylate cyclase system (Koike et al., 1995b). Koike et al. (1995b) reported that the responses to norepinephrine were resistant to the classical \( \alpha \)-adrenoceptor antagonist phentolamine and the classical \( \beta \)-adrenoceptor antagonist propranolol. In the present study, the responses to epinephrine were also resistant to phentolamine and propranolol. These results suggest that the relaxant response to epinephrine in this preparation may not be mediated by classical \( \alpha \)- and \( \beta \)-adrenoceptors.

Bupranolol is reported to be a putative probe for the presence of \( \beta \)-adrenoceptors in heart (Kaumann, 1989), digestive tract (Horinouchi and Koike, 1999a; 1999b) and adipose tissues (Langin et al., 1991; Blin et al., 1994). It is demonstrated that in low concentration (nM), bupranolol has the characterization of a non-selective \( \beta \)- and \( \beta \)-adrenoceptor antagonist and that, in high concentration (mM), bupranolol has the characterization of a \( \beta \)-adrenoceptor antagonist in addition to that of a non-selective \( \beta \)- and \( \beta \)-adrenoceptor antagonist (Kaumann, 1989). In the present study, bupranolol produced shifts of the concentration–response curve to epinephrine. Moreover, a Schild regression carried out for bupranolol against epinephrine gave the \( pA_2 \) value of 5.87. Therefore, our present results suggest that the relaxant response to epinephrine of the guinea pig taenia caecum may be mediated by \( \beta \)-adrenoceptors.

Epinephrine increased adenosine 3', 5'-cyclic monophosphate (cyclic AMP) levels in the guinea pig taenia caecum. Bupranolol antagonized cyclic AMP accumulation by epinephrine, whereas propranolol failed to antagonize this. These results suggest that the relaxant response to epinephrine in the guinea pig taenia caecum involves the adenylate cyclase system and support the notion that the response to epinephrine is mediated by \( \beta \)-adrenoceptors.

In conclusion, the main objective of the present studies was to clarify whether \( \beta \)-subtype is involved in the \( \beta \)-adrenoceptor-mediated relaxation induced by epinephrine in the guinea pig taenia caecum. Our results suggest that the relaxant response to epinephrine in the guinea pig taenia caecum is mainly mediated by \( \beta \)-adrenoceptor-induced stimulation and activation of the adenylate cyclase system.

References


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