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Possible mechanisms of β-adrenoceptor-mediated relaxation induced by noradrenaline in guinea pig taenia caecum

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Abstract

The mechanisms of the β-adrenoceptor-mediated relaxation induced by noradrenaline in guinea pig taenia caecum were investigated. Noradrenaline caused graded relaxation of this preparation. However, the concentration-response curves for noradrenaline were unaffected by propranolol ($\sim 10^{-5}$ M) or phentolamine ($\sim 10^{-5}$ M). The responses to noradrenaline were antagonized in a concentration-dependent manner by bupranolol, and Schild plots of the data revealed a pA_2 value of 5.53. Also, bupranolol antagonized responses to isoprenaline, and Schild plots of the data revealed the pA_2 value to be 8.53. Noradrenaline significantly increased the cyclic AMP level in this preparation. Bupranolol (10^{-4} M) significantly decreased the cyclic AMP response elicited by noradrenaline, whereas propranolol (10^{-5} M) produced no effect. These results suggest that the relaxant response to noradrenaline in guinea pig taenia caecum is mainly mediated by β_3-adrenoceptors (or atypical β-adrenoceptors) and that in guinea pig taenia caecum noradrenaline behaves as a β_3-selective adrenoceptor agonist.

Keywords: β-Adrenoceptor, atypical; Taenia caecum, guinea-pig; Muscle relaxation; Noradrenaline

1. Introduction

It is generally accepted that catecholamines, e.g. noradrenaline, can produce relaxation of gastrointestinal smooth muscle by an action on postjunctional α- and β-adrenoceptors (Burnstock and Wong, 1981; Bülbring and Tomita, 1987). However, it has been reported that relaxant responses to catecholamines are resistant to blockade of classical α- and β-adrenoceptors in rat distal colon (McLaughlin and MacDonald, 1990), rat gastric fundus (McLaughlin and MacDonald, 1991) and rat jejunum (MacDonald et al., 1994). The β-adrenoceptors were originally subclassified by Lands et al. (1967a,b) into β_1- and β_2-subtypes and another subtype, the β_3-adrenoceptor (atypical β-adrenoceptor), has recently been reported (Arch, 1989; Emorine et al., 1989, Emorine et al., 1994).

Our own previous studies with the guinea pig taenia caecum showed that isoprenaline-induced relaxation was predominantly mediated by β_2-adrenoceptors and that CGP 12177 (a β_1/β_2-adrenoceptor antagonist and a β_3-adrenoceptor agonist)-induced relaxation was solely mediated through β_3-adrenoceptors (Koike et al., 1994).

In the present study we found that the relaxant response to noradrenaline, a neurotransmitter, was resistant to blockade by propranolol in the guinea pig taenia caecum. Therefore, its mechanisms of action were investigated.

2. Materials and methods

2.1. Mechanical responses

Male guinea pigs weighing 300–500 g were killed by a blow on the head. A 2–3-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; $CaCl_2$, 2.2; $MgCl_2$, 2.1; $NaHCO_3$, 5.9 and glucose, 2.8 mM) kept at 32° C and bubbled with a mixture of 95% O_2 and 5% CO_2. 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 of the agonists

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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, a 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 were without the tension 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. Six or more concentration-response curves could be made in succession. The agonistic potency was expressed as the pD_2 value (Van Rossum, 1963). The competitive antagonistic potency was expressed as the pA_2 value, and was calculated according to the method of Tallarida et al. (1979), which was originally reported by Arunlakshana and Schild (1959).

2.2. Estimation of cyclic AMP levels

Male guinea pigs weighing 300–500 g were killed by a blow on the head. The guinea pig taenia caeci were carefully dissected and suspended in Ringer-Locke solution (NaCl, 154; KCl, 5.6; $CaCl_2$, 2.2; $MgCl_2$, 2.1; $NaHCO_3$, 5.9 and glucose, 2.8 mM). 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% O_2 and 5% CO_2. 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 was used for estimating any change in 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) which contained 0.5 pmol of [^{3}H]cyclic GMP to estimate the recovery of cyclic AMP (Ohkubo et al., 1976). The homogenate was centrifuged at 3000 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 and for calculating the recovery (Ohkubo et al., 1976). The quantity of cyclic AMP was determined by a radioimmunoassay, using a commercial kit (Yamasa, Chiba, Japan). The radioimmunoassay for cAMP was carried out in 0.05 M sodium acetate buffer, pH 6.2. Assays were performed in non-siliconized disposable culture tubes. Each tube contained 100 μl of cAMP standard or unknown solution, 100 μl of antibody and 100 μl of the ^{125}I-cAMP in a final volume of 500 μl. The reaction mixture was then incubated 18–24 h at 4° C. Activated carbon was used for separating bound and free ^{125}I-ligand before centrifugation at 4° C for 15 min, and the precipitate was counted in a gamma spectrometer (Aloka ARC-370M, Tokyo, Japan).

2.3. Protein assay

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

2.4. Data analysis

Numerical results are expressed as means ± S.E. and statistical analyses were performed using Student's *t*-test and Duncan's new multiple range test as appropriate. A P value of less than 0.05 was considered a significant difference.

2.5. Drugs and chemicals

The drugs used were obtained from the following sources: (−)-isoprenaline hydrochloride, (−)-noradrenaline bitartrate, butoxamine hydrochloride, (±)-propranolol hydrochloride, reactive blue 2 (Sigma Chemical Co., St. Louis, MO, USA); bupranolol hydrochloride (Looser; Kaken Seiyaku Co., Tokyo, Japan); atenolol, tetraethylammonium chloride, glibenclamide (Research Biochemicals, Natick, MA, USA); prazosin hydrochloride, yohimbine hydrochloride (Wako Pure Chemical Industries, Osaka, Japan); phentolamine mesylate (Ciba Geigy, Basel, Switzerland) and [^{3}H]cyclic GMP (specific activity, 36.4 Ci/mmol; 1 Ci = 37 GBq; New England Nuclear, Boston, MA, USA). All the drugs were used as solutions in distilled water. The other chemicals used were of analytical grade.

3. Results

3.1. Responses to noradrenaline

Noradrenaline caused graded relaxation of the guinea pig taenia caecum in which the tone had been raised, with a pD_2 value of 6.62 ± 0.05 (n = 6) (Fig. 1). The concentration-response curves for noradrenaline were unaffected by propranolol ($\sim 10^{-5}$ M, Fig. 1) or phentolamine ($\sim 10^{-5}$ M, Fig. 2). Propranolol (10^{-5} M) reduced smooth muscle tone by itself (Fig. 1). Atenolol ($\sim 3 \times 10^{-4}$ M, a selective β_1-adrenoceptor antagonist), butoxamine ($\sim 10^{-4}$ M, a selective β_2-adrenoceptor antagonist), prazosin ($\sim 10^{-5}$ M, a selec-

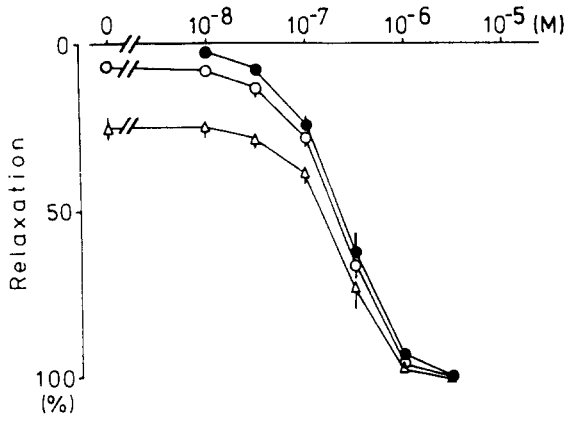

Fig. 1. Effect of propranolol on the concentration-response curve for noradrenaline in the guinea pig taenia caecum. Control (●), propranolol 10^{-6} M (○), 10^{-5} M (△). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline (3×10^{-7} M), and abscissa: concentration (M) of noradrenaline. Each point is presented as a mean ± S.E. of six experiments.

tive α_1-adrenoceptor antagonist), yohimbine (~ 10^{-5} M, a selective α_2-adrenoceptor antagonist), reactive blue 2 (~ 10^{-4} M, a selective P_{2y}-purinoceptor antagonist), tetraethylammonium (~ 10^{-2} M, a voltage-dependent K^+ channel blocker) and glibenclamide (~ 10^{-5} M, an ATP-dependent K^+ channel blocker) had no effect on the potency of noradrenaline (data not shown). Moreover, desmethylimipramine (~ 10^{-7} M, a neuronal uptake inhibitor) and normetanephrine (~ 10^{-6} M, an extraneuronal uptake inhibitor) had no effect on the potency of noradrenaline (data not shown).

3.2. Effects of bupranolol on responses to noradrenaline and isoprenaline

The responses to noradrenaline were antagonized in a concentration-dependent manner by bupranolol (Fig. 3A). The Schild plot (Fig. 3B) of the data revealed the pA_2 value to be 5.53 ± 0.06, the slope of the regression line (0.94 ± 0.09) not being significantly different from unity. Also, bupranolol antagonized responses to isoprenaline in a concentration-dependent manner (Fig. 4A). Schild plot (Fig. 4B) of the data revealed the pA_2 value to be 8.53 ± 0.04, the slope of the regression line (1.13 ± 0.09) not being significantly different from unity. The difference between the pA_2 values for bupranolol against noradrenaline and isoprenaline was statistically significant ($P < 0.05$). Bupranolol itself had no effect on the degree of tone.

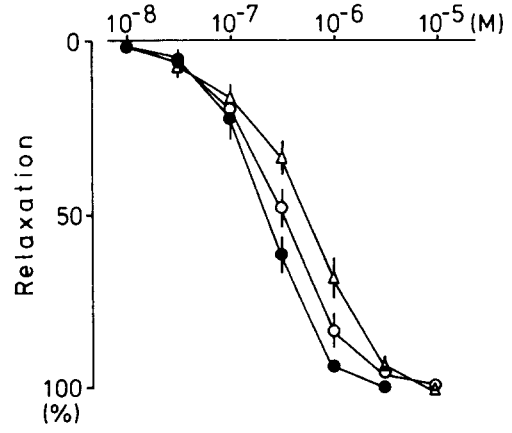

Fig. 2. Effect of phentolamine on the concentration-response curve for noradrenaline in the guinea pig taenia caecum. Control (●), phentolamine 10^{-6} M (○), 10^{-5} M (△). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of noradrenaline. Each point is presented as a mean ± S.E. of six experiments.

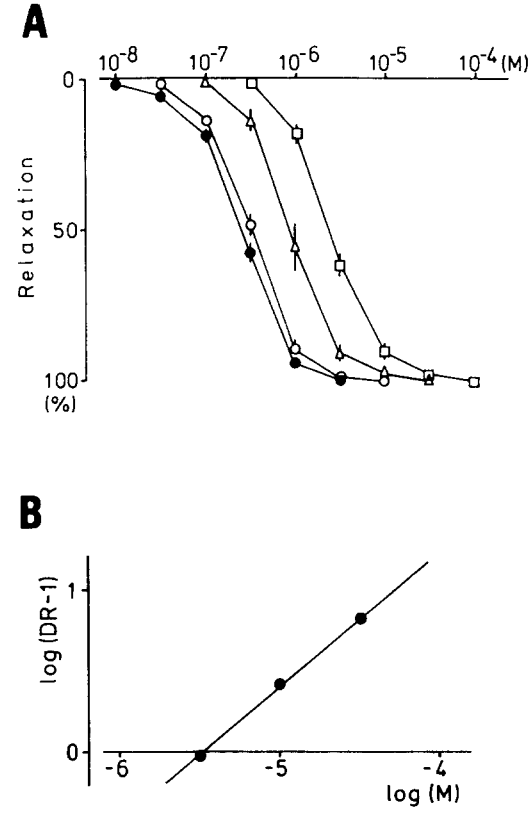

Fig. 3. Determination of the pA_2 value for bupranolol against noradrenaline. (A) Antagonism of noradrenaline-induced relaxation by bupranolol. Control (●), bupranolol 3×10^{-6} M (○), 10^{-5} M (△), 3×10^{-5} M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of noradrenaline. Each point is presented as a mean ± S.E. of six experiments. (B) Schild plot for antagonism of noradrenaline by bupranolol. The data are taken from experiments shown in (A).

3.3. Effects of propranolol and bupranolol on cyclic AMP levels

The maximal concentration (3×10^{-7} M) of isoprenaline increased the cyclic AMP level in the guinea pig taenia caecum (control, 3.11 ± 0.08 pmol/mg protein; with isoprenaline, 5.21 ± 0.28 pmol/mg protein). When the guinea pig taenia caecum was incubated with

noradrenaline (3×10^{-5} M) for 2 min, the cyclic AMP level (4.82 ± 0.24 pmol/mg protein) was increased 1.55-fold ($P < 0.05$) over that of the control (Fig. 5). When taenia caecum was incubated with bupranolol (10^{-4} M) in combination with noradrenaline, the cyclic AMP level (3.43 ± 0.11 pmol/mg protein) was significantly decreased ($P < 0.05$) (Fig. 5). However, propranolol (10^{-5} M) produced no effect (4.75 ± 0.24 pmol/mg protein) (Fig. 5).

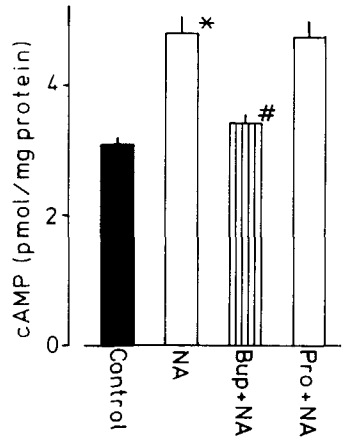

Fig. 5. Effects of bupranolol and propranolol on the noradrenaline-induced cyclic AMP level in the guinea pig taenia caecum. Control; basal level, NA; noradrenaline (3×10^{-5} M), Bup; bupranolol (10^{-4} M), Pro; propranolol (10^{-5} M). Each column is presented as a mean ± S.E. of six experiments. The asterisk (*) denotes a significant difference from control ($P < 0.05$), and the sharp (#) denotes a significant difference from noradrenaline alone ($P < 0.05$).

4. Discussion

It was initially thought that the β_1- or β_2-adrenoceptor subtypes modulate all the effects of catecholamines. As previously reported, the isoprenaline (a catecholamine)-induced relaxant response in the guinea pig taenia caecum is mainly mediated through β_2-adrenoceptors (Koike et al., 1994). In this study, however, the responses to noradrenaline (a catecholamine) were resistant to the classical α-adrenoceptor antagonist phentolamine and the classical β-adrenoceptor antagonist propranolol. Moreover, the selective α_1-, α_2-, β_1- and β_2-adrenoceptor antagonists, prazosin, yohimbine, atenolol and butoxamine, respectively, produced no effect. These results suggest that the postjunctional inhibitory response to noradrenaline in this preparation may not be mediated by classical α- and β-adrenoceptors.

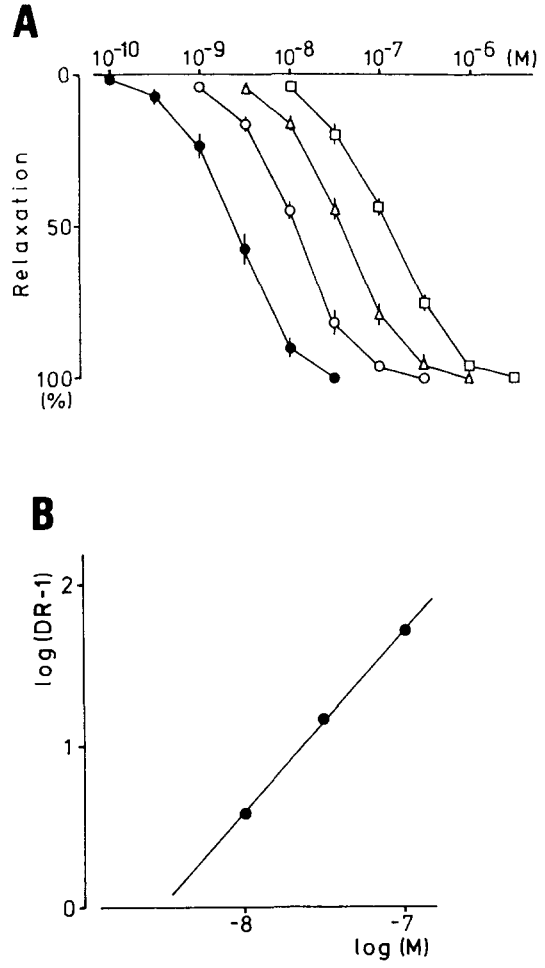

Fig. 4. Determination of the pA_2 value for bupranolol against isoprenaline. (A) Antagonism of isoprenaline-induced relaxation by bupranolol. Control (●), bupranolol 10^{-8} M (○), 3×10^{-8} M (△), 10^{-7} M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of isoprenaline. Each point is presented as a mean ± S.E. of six experiments. (B) Schild plot for antagonism of isoprenaline by bupranolol. The data are taken from experiments shown in (A).

It is well known that P_{2y}-purinoceptors mediate relaxation in the guinea pig taenia caecum (Burnstock and Kennedy, 1985) and that in this muscle noradrenaline causes hyperpolarization of the membrane by increasing K^+ conductance and stopping spontaneous activity, resulting in relaxation (Bülbring and Tomita, 1987). In the present study, the relaxation in response to noradrenaline was elicited in the presence of a P_{2y}-purinoceptor antagonist reactive blue 2, a voltage-dependent K^+ channel blocker tetraethylammonium and an ATP-dependent K^+ channel blocker glibenclamide. These results rule out the involvement of P_{2y}-purinoceptors and K^+ channels in the relaxant responses to noradrenaline.

In the present study bupranolol produced shifts of the concentration-response curves for noradrenaline and isoprenaline. Moreover, a Schild regression analysis carried out for bupranolol against noradrenaline gave a pA_2 value of 5.53, which was significantly different from the value (8.53) obtained with isoprenaline as agonist. Bupranolol is reported to be a puta-

tive probe for the presence of β_3-adrenoceptors (or atypical β-adrenoceptor) in heart (Kaumann, 1989), digestive tract and adipose tissues (Langin et al., 1991; Blin et al., 1994). Kaumann (1989) reported that the concentration-response curves for the stimulant effects of (−)-pindolol, a β-adrenoceptor partial agonist, on atria were biphasic, with EC_{50} values around 1 nM and 100 nM. Further, they reported that the high-sensitivity component of pindolol was mediated through both β_1- and β_2-adrenoceptors because it was blocked by the classical antagonist propranolol, by a low concentration (nM) of bupranolol and by antagonists selective for β_1- and β_2-adrenoceptors, and that the low-sensitivity component of pindolol was not blocked by propranolol, was resistant to blockade by selective β_1- and β_2-adrenoceptor antagonists but was blocked by a high concentration (μM) of bupranolol. It has been suggested that at low concentrations bupranolol is a non-selective β_1- and β_2-adrenoceptor antagonist and that at high concentrations bupranolol is a selective atypical (β_3)-adrenoceptor antagonist. Therefore, our present results suggest that the relaxant response to noradrenaline of the guinea pig taenia caecum may be mediated by β_3-adrenoceptors (or atypical β-adrenoceptors).

Noradrenaline increased adenosine 3′,5′-cyclic monophosphate (cyclic AMP) levels in the guinea pig taenia caecum. Bupranolol antagonized the cyclic AMP accumulation elicited by noradrenaline whereas, propranolol did not. In general, all three β-adrenoceptor subtypes appear to be linked to adenylate cyclase activation (Tate et al., 1991). These results suggest that the relaxant response to noradrenaline in the guinea pig taenia caecum involves the adenylate cyclase system and support the notion that the response to noradrenaline is mediated by β_3-adrenoceptors (or atypical β-adrenoceptors).

In conclusion, this pharmacological analysis shows that the relaxant response to noradrenaline in the guinea pig taenia caecum is mainly mediated by β_3-adrenoceptor (or atypical β-adrenoceptor)-induced stimulation and activation of the adenylate cyclase system and suggests that in the guinea pig taenia caecum noradrenaline behaves as a β_3-selective adrenoceptor agonist.

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