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European Journal of Pharmacology 334 (1997) 217–221

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β_3-adrenoceptors mediate relaxation of guinea pig taenia caecum by BRL37344A and BRL35135A

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Received 10 April 1997; revised 7 July 1997; accepted 11 July 1997

Abstract

β-Adrenoceptor-mediated relaxation of guinea pig taenia caecum was investigated by studying the effects of the β_3-adrenoceptor agonists, BRL37344A [(R^*,R^*)-$(\pm)$-4-[2′-{2-hydroxy-2-(3-chlorophenyl) ethylamino} propyl] phenoxyacetic acid sodium salt sesquihydrate] and BRL35135A [(R^*,R^*)-$(\pm)$-methyl-4-[2-{2-hydroxy-2-(3-chlorophenyl) ethylamine} propyl] phenoxyacetate hydrobromide]. BRL37344A and BRL35135A caused dose-dependent relaxation of the guinea pig taenia caecum. The concentration–response curves for BRL37344A and BRL35135A were unaffected by propranolol, ICI118551 [erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol], atenolol, butoxamine, prazosin, yohimbine and phentolamine. Bupranolol produced shifts of the concentration–response curves for BRL37344A and BRL35135A. Schild regression analyses carried out for bupranolol against BRL37344A and BRL35135A gave pA_2 values of 5.79 and 5.84, respectively. These results suggest that the relaxant response to BRL37344A and BRL35135A of the guinea pig taenia caecum is mediated by β_3-adrenoceptors. © 1997 Elsevier Science B.V.

Keywords: β-Adrenoceptor; β_3-Adrenoceptor; BRL37344A, BRL35135A; Taenia caecum, guinea pig; Muscle relaxation

1. Introduction

β-Adrenoceptors were initially classified as β_1- or β_2-adrenoceptor subtypes by Lands et al. (1967). During the past few years, both pharmacological and molecular studies have revealed that β-adrenoceptors are more heterogeneous than believed thus far. In a number of tissues, especially adipose and gastrointestinal tissue, responses appeared to be mediated by a receptor with distinct characteristics, different from classical β_1- and/or β_2-adrenoceptors (Arch and Kaumann, 1993). Cloning and characterization of a β-adrenoceptor with atypical properties, from a human genomic library, demonstrated conclusively the existence of a third subtype of β-adrenoceptor designated as β_3-adrenoceptor (Emorine et al., 1989). The responses mediated by β_3-adrenoceptors are characterized by resistance to blockade by classical β_1- and β_2-adrenoceptor antagonists, low stereoselectivity ratios to antagonist enantiomers, and activation by novel β-adrenoceptor agonists (Zaagsma and Nahorski, 1990).

We had demonstrated that 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,b), and that β_1-adrenoceptors are not involved (Koike et al., 1994).

Selective agonists for β_3-adrenoceptors, notably BRL26830, BRL35135A [(R^*,R^*)-$(\pm)$-methyl-4-[2-{2-hydroxy-2-(3-chlorophenyl) ethylamine} propyl] phenoxyacetate hydrobromide] and their respective acid metabolites, BRL28410 and BRL37344A [(R^*,R^*)-$(\pm)$-4-[2′-{2-hydroxy-2-(3-chlorophenyl) ethylamino} propyl] phenoxyacetic acid sodium salt sesquihydrate], were first described by Arch et al. (1984). The strongest evidence for the existence of β_3-adrenoceptors came from studies with a series of novel agonists (e.g., BRL37344A, BRL35135A) that exert potent and selective thermogenic and anti-obesity activities in animal models (Wilson et al., 1984; Arch et al., 1984; Arch, 1989). Therefore, we have studied in detail the β-adrenoceptor-mediated relaxation of guinea

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pig taenia caecum by measuring the potencies of BRL37344A and BRL35135A.

2. Materials and methods

2.1. 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; $CaCl_2$, 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 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. Agonistic potency was expressed as the pD_2 value (Van Rossum, 1963). The competitive antagonistic potency 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).

2.2. Data analysis

Numerical results are 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.

2.3. Drugs

The drugs used were obtained from the following sources: isoprenaline hydrochloride, butoxamine hydrochloride, propranolol hydrochloride (Sigma Chemical, St. Louis, MO); bupranolol hydrochloride (Looser; Kaken Seiyaku, Tokyo); atenolol, ICI118551 [erythro-1-(7-methylindan-4-yloxy)-3-(isopropylamine)-butan-2-ol] (Research Biochemicals, Natik, MA); prazosin hydrochloride, yohimbine hydrochloride (Wako Pure Chemical, Osaka); phentolamine mesylate (Ciba Geigy, Basel); BRL37344A [(R^*,R^*)-(±)-4-[2′-{2-hydroxy-2-(3-chlorophenyl) ethylamino} propyl] phenoxyacetic acid sodium salt sesquihydrate], BRL35135A [(R^*,R^*)-(±)-methyl-4-[2-{2-hy-

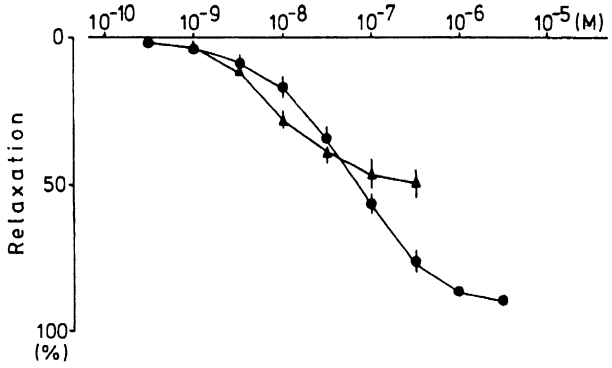

Fig. 1. Concentration–response curves to BRL37344A (●) and BRL35135A (▲). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline (3×10^{-7} M), and abscissa: concentration (M) of the test drugs. Each point represents the mean ± S.E. of six experiments.

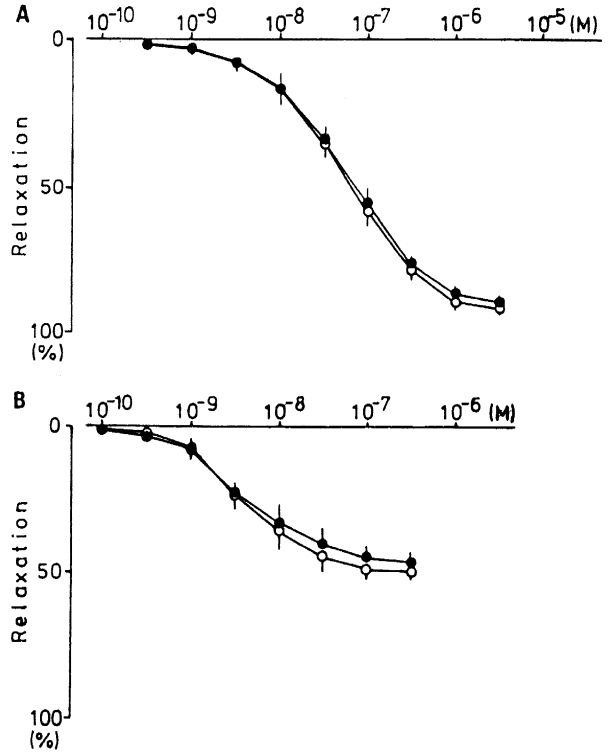

Fig. 2. Effects of propranolol on the concentration–response curves for BRL37344A (A) and BRL35135A (B). Control (●), propranolol 10^{-5} M (○). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of BRL37344A or BRL35135A. Each point represents the mean ± S.E. of six experiments.

droxy-2-(3-chlorophenyl) ethylamine} propyl] phenoxyacetate hydrobromide] (Smith Kline Beecham Pharmaceuticals, Surrey). All the drugs were used as a solution in distilled water. The other chemicals used were of analytical grade.

3. Results

3.1. Responses to BRL37344A and BRL35135A

BRL37344A and BRL35135A caused a dose-dependent relaxation of the guinea pig taenia caecum piece in which the tone had been raised (Fig. 1). The intrinsic activity and the pD_2 value of BRL37344A were 0.89 ± 0.01 and 7.27 ± 0.07, respectively, and those of BRL35135A were 0.49 ± 0.05 and 8.09 ± 0.05, respectively.

3.2. Resistance to blockade by β-adrenoceptor antagonists

The concentration–response curves for BRL37344A and BRL35135A were unaffected by propranolol ($\sim 10^{-5}$ M, Fig. 2) or ICI118551 ($\sim 10^{-5}$ M, a selective β_2-adrenoceptor antagonist, Fig. 3). 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 selective α_1-adrenoceptor antagonist), yohimbine ($\sim 10^{-5}$ M, a selective α_2-adrenoceptor antagonist) and phentolamine ($\sim 10^{-5}$ M) had no effect on the potencies of BRL37344A and BRL35135A (data not shown).

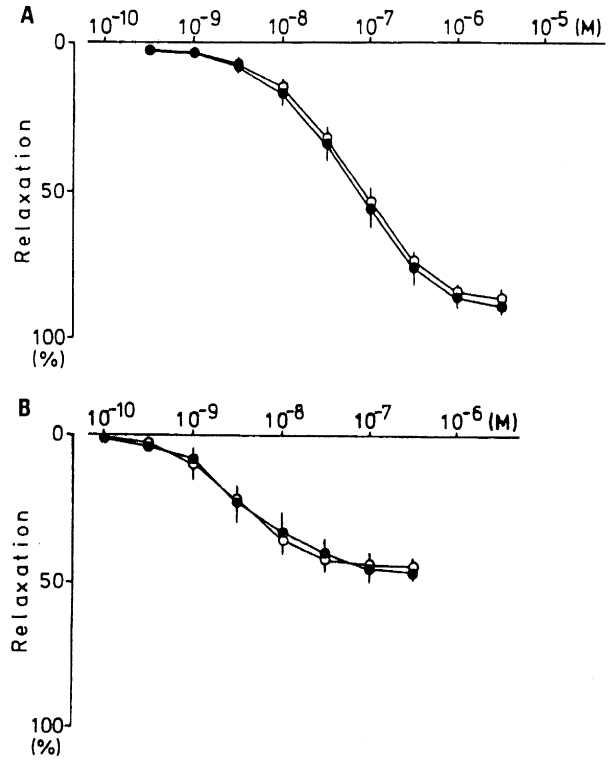

Fig. 3. Effects of ICI118551 on the concentration–response curves for BRL37344A (A) and BRL35135A (B). Control (●), ICI118551 10^{-5} M (○). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of BRL37344A or BRL35135A. Each point represents the mean ± S.E. of six experiments.

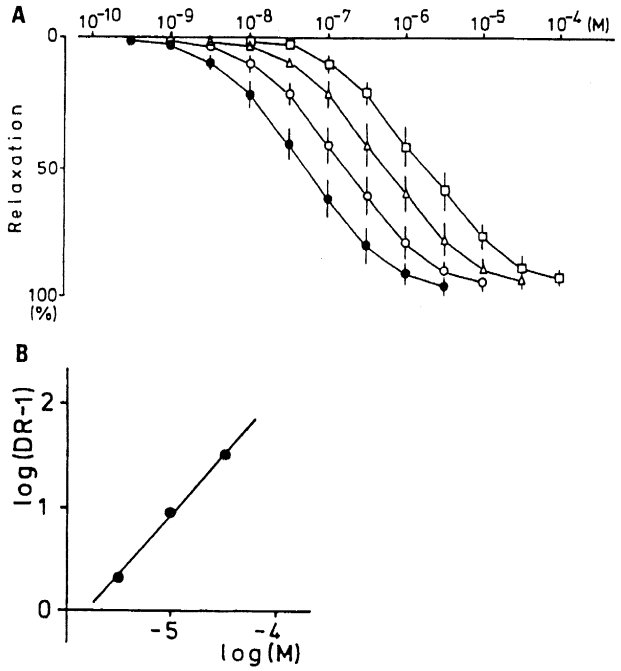

Fig. 4. Determination of the pA_2 value for bupranolol against BRL37344A. (A) Antagonism of BRL37344A-induced relaxation by bupranolol. Control (●), bupranolol 3×10^{-6} M (○), 10^{-5} M (△), 3×10^{-5} M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of BRL37344A. Each point represents the mean ± S.E. of six experiments. (B) Schild plot for antagonism of BRL37344A by bupranolol. The data are taken from experiments shown in (A).

3.3. Antagonism by bupranolol

The responses to BRL37344A were antagonized in a concentration-dependent manner by bupranolol (Fig. 4A). The Schild plot (Fig. 4B) of the data revealed the pA_2 value to be 5.79 ± 0.11, the slope of the regression line (1.08 ± 0.06) not being significantly different from unity. Also, bupranolol antagonized the responses to BRL35135A in a concentration-dependent manner (Fig. 5A). A Schild plot (Fig. 5B) of the data revealed the pA_2 value to be 5.84 ± 0.14, the slope of the regression line (1.06 ± 0.04) not being significantly different from unity.

4. Discussion

Experiments with BRL37344A or BRL35135A have suggested the presence of atypical β-adrenoceptors, or β_3-adrenoceptors, mediating relaxation in several gut preparations, for example, guinea pig ileum (Bond and

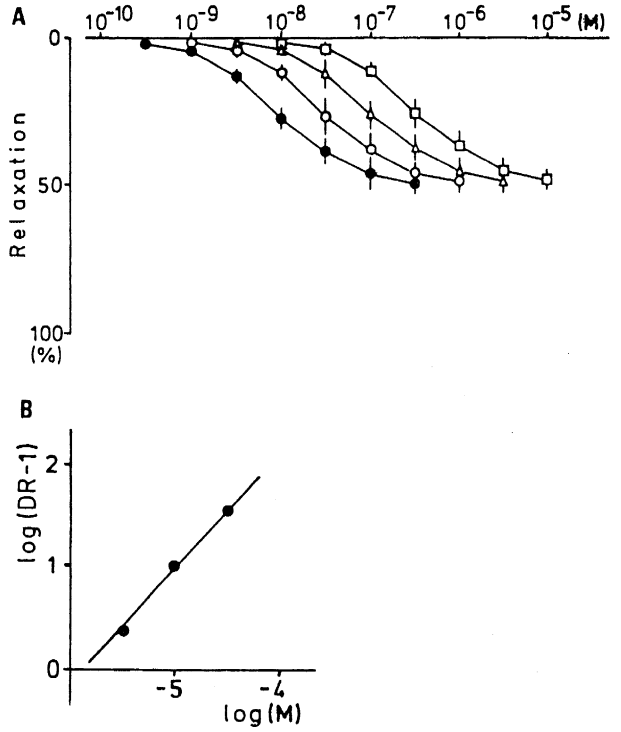

Fig. 5. Determination of the pA_2 value for bupranolol against BRL35135A. (A) Antagonism of BRL35135A-induced relaxation by bupranolol. Control (●), bupranolol 3×10^{-6} M (○), 10^{-5} M (△), 3×10^{-5} M (□). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of BRL35135A. Each point represents the mean ± S.E. of six experiments. (B) Schild plot for antagonism of BRL35135A by bupranolol. The data are taken from experiments shown in (A).

Clarke, 1988), rat distal colon (McLaughlin and MacDonald, 1990), rat gastric fundus (McLaughlin and MacDonald, 1991), rat ileum (Growcott et al., 1993) and rat oesophageal muscularis mucosae (De Boer et al., 1993).

In our present study, BRL37344A and BRL35135A relaxed the guinea pig taenia caecum and behaved as partial agonists with intrinsic activities of 0.89 and 0.49, respectively. Other workers have noted that BRL37344A may not always produce a full agonist response when compared to isoprenaline. For example, in human adipocytes, BRL37344A was found to be significantly less efficacious than isoprenaline to cause lipolysis whereas, in rat adipocytes, each agent possessed similar efficacy (Hollenga et al., 1990). Furthermore, in rat jejunum, where effects on resting tone were measured, BRL37344A produced a significantly lower maximum response than did isoprenaline (Van der Vliet et al., 1990). Wheeldon et al. (1994) have reported that the ester, BRL35135A, which is completely demethylated to BRL37344A in vivo, causes tachycardia. Fig. 1 shows that BRL35135A is a partial agonist and BRL37344A an almost full agonist. Furthermore, the effective concentration ranges of the two agonists, BRL37344A and BRL35135A, are similar. These results show that BRL35135A acts directly, thus not after hydrolysis to BRL37344A, under the present in vitro conditions. Then, the responses to BRL37344A and BRL35135A in the guinea pig taenia caecum were resistant to propranolol, atenolol, butoxamine, phentolamine, prazosin and yohimbine. As previously reported, the relaxant response in the guinea pig taenia caecum is predominantly mediated through β_2-adrenoceptors (Koike et al., 1994). In the present study, the selective β_2-adrenoceptor antagonist, ICI118551, produced no effect. These results suggest that the relaxant responses to BRL37344A and BRL35135A are not mediated by classical α- and β-adrenoceptors.

Bupranolol was used in our study since it acts as an antagonist at β_3-adrenoceptors (Arch and Kaumann, 1993; Blin et al., 1993), although at concentrations much higher than those necessary for the blockade of β_1- or β_2-adrenoceptors (Kaumann, 1996; Kaumann and Molenaar, 1996; Malinowska and Schlicker, 1996). Bupranolol shifted the concentration–response curves for BRL37344A and BRL35135A. Schild regression analyses carried out for bupranolol against BRL37344A and BRL35135A gave pA_2 values of 5.79 and 5.84, respectively. 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). Our present results suggest that the relaxant response of the guinea pig taenia caecum to BRL37344A and BRL35135A may be mediated by β_3-adrenoceptors.

For an effect to be considered as mediated through β_3-adrenoceptors Arch and Kaumann (1993) proposed three criteria: (i) the receptor should be selectively stimulated by β_3-receptor-selective agonists (e.g., BRL37344A), (ii) the receptor should be stimulated by non-conventional partial agonists (e.g., CGP12177) and (iii) the receptor should be resistant to blockade by antagonists possessing only high affinity for β_1- and β_2-adrenoceptors. Our own previous studies with the guinea pig taenia caecum showed that CGP12177-induced relaxation is not influenced by the addition of β- and α-adrenoceptor antagonists, and that CGP12177-induced relaxation is solely mediated through β_3-adrenoceptors (Koike et al., 1995c). Therefore, all three criteria were fulfilled for the agonist-evoked relaxation of the guinea pig taenia caecum.

We conclude that the present pharmacological experiments showed that the relaxant responses to BRL37344A and BRL35135A in the guinea pig taenia caecum are mediated by β_3-adrenoceptors.

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