The β_3 -Adrenoceptor-Mediated Relaxation Induced by Dopamine in Guinea Pig Taenia Caecum

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Abstract

The mechanisms of the β -adrenoceptor-mediated relaxation induced by dopamine in guinea pig taenia caecum were examined. The relaxant response to dopamine was unaffected by propranolol (10^{-8} – 10^{-5} M) or phentolamine (10^{-8} – 10^{-5} M). Atenolol (3×10^{-7} – 3×10^{-4} M), butoxamine (10^{-7} – 10^{-4} M), prazosin (10^{-8} – 10^{-5} M), yohimbine (10^{-8} – 10^{-5} M), SCH 23390 (10^{-8} – 10^{-5} M) and haloperidol (10^{-8} – 10^{-5} M) had no effect on the potency of dopamine. The response to dopamine was antagonized in a concentration-dependent manner by bupranolol (3×10^{-6} – 3×10^{-5} M), and Schild plot of the data revealed the pA_2 value of 5.55 and the slope of the regression line was 1.13. These results suggest that the relaxant response to dopamine in the guinea pig taenia caecum is mainly mediated by the β_3 -adrenoceptors.

Key words: β_3 -adrenoceptor, dopamine, 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, salbutamol and fenoterol, which belong to the aryethanolamine class, are mediated by both the β_2 - and the β_3 -adrenoceptors in the guinea pig

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taenia caecum (Koike *et al.*, 1997; Akimoto *et al.*, 2002). On the other hand, the relaxant responses to noradrenaline and adrenaline, the neurotransmitters, in the guinea pig taenia caecum are mainly mediated by the β_3 -adrenoceptors (Koike *et al.*, 1995a; Koike *et al.*, 2000). Then, we have doubt whether the other endogenous catecholamine, for example dopamine, causes relaxation of the guinea pig taenia caecum mediating through the β_3 -adrenoceptors.

Dopamine (3,4-dihydroxyphenylethylamin) is the immediate metabolic precursor of noradrenaline and adrenaline. In addition, dopamine is a central neurotransmitter particularly important in the regulation of movement and possesses important intrinsic pharmacological properties. Moreover, dopamine exerts a positive inotropic effect on the myocardium, acting on the β_1 -adrenoceptors (Hoffman, 2001). However, it is not known whether the β_3 -subtype is involved in the β -adrenoceptor-mediated relaxation induced by dopamine. Therefore, the aim of this study is to examine the mode of the action of dopamine 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 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 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. Concentration-response curves for isoprenaline (●), adrenaline (○), noradrenaline (△) and dopamine (□) in the guinea pig taenia caecum. Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of the test drugs. Each point represents the mean $\pm$ S.E.M. of six experiments.

Drugs

The drugs used were obtained from the following sources: dopamine hydrochloride, isoprenaline hydrochloride, noradrenaline bitartrate, adrenaline bitartrate, butoxamine hydrochloride, propranolol hydrochloride, SCH 23390 hydrochloride (7-chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3-benzazepine), haloperidol (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.); prazosin hydrochloride, yohimbine hydrochloride (Wako Pure Chemical 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

Dopamine caused graded relaxation of the guinea pig taenia caecum piece in which the tone had been raised spontaneously, with the pD_2 value of 4.52 ± 0.10 (Fig. 1). Isoprenaline, noradrenaline and adrenaline all caused graded relaxation of the guinea pig taenia caecum (Fig. 1). The pD_2 values and intrinsic activities of these drugs are summarized in Table 1.

Propranolol (10^{-8} – 10^{-5} M), a nonselective β_1 - and β_2 -adrenoceptor antagonist, did not significantly affect the relaxant response to dopamine (Fig. 2). Moreover, phentolamine (10^{-8} – 10^{-5} M), a nonselective α_1 - and α_2 -adrenoceptor antagonist, did not significantly affect the relaxant response to dopamine (data not shown). Atenolol (3×10^{-7} – 3×10^{-4} M, a selective β_1 -adrenoceptor antagonist), butoxamine (10^{-7} – 10^{-4} M, a selective β_2 -adrenoceptor antagonist), prazosin (10^{-8} – 10^{-5} M, a selective α_1 -adrenoceptor antagonist), yohimbine (10^{-8} – 10^{-5} M, a

Table 1 The pD_2 values and intrinsic activities of catecholamines

	pD_2 value	i.a.
Isoprenaline	8.35 ± 0.10	1.00
Adrenaline	7.36 ± 0.06	0.98 ± 0.05
Noradrenaline	6.61 ± 0.09	0.97 ± 0.06
Dopamine	4.52 ± 0.10	0.94 ± 0.04

Each value represents the mean $\pm$ S.E.M. of six experiments.

Fig. 2. Effect of propranolol on the concentration-response curve for dopamine in the guinea pig taenia caecum. Control (●), propranolol 10^{-5} M (○). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of dopamine. Each point represents the mean $\pm$ S.E.M. of six experiments.

selective α_2 -adrenoceptor antagonist), SCH 23390 (10^{-8} – 10^{-5} M, a selective D_1 -dopamine receptor antagonist) and haloperidol (10^{-8} – 10^{-5} M, a selective D_2 -dopamine receptor antagonist) had no effect on the potency of dopamine (data not shown).

Bupranolol (3×10^{-6} – 3×10^{-5} M) caused competitive antagonism of the relaxant response to dopamine (Fig. 3A). The Schild plot (Fig. 3B) of the data gave the pA_2 value of 5.55 ± 0.10 and the slope of the regression line (1.13 ± 0.11) was not significantly different from unity. The pA_2 values and Schild slopes for bupranolol against noradrenaline, adrenaline and dopamine are summarized in Table 2.

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). Koike *et al.* (1995b; 2000) reported that the responses to noradrenaline and

Fig. 3. Determination of the pA_2 value for bupranolol against dopamine in the guinea pig taenia caecum. A: Antagonism of dopamine-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 dopamine. Each point represents the mean $\pm$ S.E.M. of six experiments. B: Schild plot for antagonism of dopamine by bupranolol. The data are taken from experiments shown in A.

Table 2 The pA_2 values and Schild slopes for bupranolol against noradrenaline, adrenaline and dopamine

	Noradrenaline	pA_2 value (Slope) Adrenaline	Dopamine
Bupranolol	$5.53 \pm 0.06^{(1)}$ (0.94 ± 0.09)	$5.87 \pm 0.04^{(2)}$ (1.10 ± 0.05)	5.55 ± 0.10 (1.13 ± 0.11)

Each value represents the mean $\pm$ S.E.M. of six experiments. ¹⁾ Values from Koike *et al.*, 1995. ²⁾ Values from Koike *et al.*, 2000.

adrenaline 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. In the present study, the responses to dopamine were also resistant to phentolamine, propranolol, prazosin, yohimbine, atenolol, butoxamine, SCH 23390 and haloperidol. These results suggest that the relaxant response to dopamine in this preparation may not be mediated by classical α - and β -adrenoceptors, and dopamine receptors.

Bupranolol is reported to be a putative probe for the presence of β_3 -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 β_1 - and β_2 -adrenoceptor antagonist and that, in high concentration (μ M), bupranolol has the characterization of a β_3 -adrenoceptor antagonist in addition to that of a non-selective β_1 - and β_2 -adrenoceptor antagonist (Kaumann, 1989). In the present study, bupranolol produced shifts of the concentration-response curve to dopamine. Moreover, a Schild regression carried out for bupranolol against dopamine gave the

pA₂ value of 5.55. The pA₂ value for bupranolol against dopamine is in good agreement with those for bupranolol against noradrenaline and adrenaline. Therefore, our present results suggest that the relaxant response to dopamine of the guinea pig taenia caecum may be mediated by the β_3 -adrenoceptors.

Previous study has suggested that the relaxant responses to isoprenaline, a non-selective β_1 - and β_2 -adrenoceptor agonist, salbutamol and fenoterol, selective β_2 -adrenoceptor agonists, which are the synthesized chemicals, in the guinea pig taenia caecum are mediated by both the β_2 - and the β_3 -adrenoceptors (Koike *et al.*, 1997; Akimoto *et al.*, 2002). On the other hand, the relaxant responses to noradrenaline and adrenaline, the neurotransmitters, are mainly mediated by the β_3 -adrenoceptors (Koike *et al.*, 1995b; Koike *et al.*, 2000) and the relaxant response to dopamine, the precursor of noradrenaline and adrenaline, is also mediated by the β_3 -adrenoceptors. These results suggest that the chemically synthesized β -adrenoceptor agonists recognize both the β_2 - and the β_3 -adrenoceptors, but that the adrenergic neurotransmitters may behave as a selective β_3 -adrenoceptor agonist.

In conclusion, our results suggest that the relaxant response to dopamine in the guinea pig taenia caecum is mainly mediated by the β_3 -adrenoceptors.

References

- Akimoto, Y., Horinouchi, T., Tanaka, Y. and Koike, K. (2002). The β_2 - and β_3 -adrenoceptor-mediated relaxation induced by fenoterol in guinea pig taenia caecum. *J. Smooth Muscle Res.* **38**: 145–151.
- Arunlakshana, O. and Schild, H.O. (1959). Some quantitative uses of drug antagonists. *Br. J. Pharmacol. Chemother.* **14**: 48–58.
- Bianchetti, A. and Manara, L. (1990). In vitro inhibition of intestinal motility by phenylethanolaminotetralines: evidence of atypical beta-adrenoceptors in rat colon. *Br. J. Pharmacol.* **100**: 831–839.
- Blin, N., Nahmias, C., Drumare, M.F. and Strosberg, A.D. (1994). Mediation of most atypical effects by species homologues of the β_3 -adrenoceptor. *Br. J. Pharmacol.* **112**: 911–919.
- De Ponti, F., Gibelli, G., Croci, T., Arcidiaco, M., Crema, F. and Manara, L. (1996). Functional evidence of atypical β_3 -adrenoceptors in the human colon, using the β_3 -selective adrenoceptor antagonist, SR59230A. *Br. J. Pharmacol.* **117**: 1374–1376.
- Hoffman, B.B. (2001). Catecholamines, sympathomimetic drugs, and adrenergic receptor antagonists. In: *The Pharmacological Basis of Therapeutics: Tenth Edition*, ed. by J.G. Hardman and L.E. Limbird, McGraw-Hill, New York, pp. 215–268.
- Horinouchi, T. and Koike, K. (1999a). Characterization of atypical β -adrenoceptor in the guinea pig duodenum. *Eur. J. Pharmacol.* **376**: 61–66.
- Horinouchi, T. and Koike, K. (1999b). Pharmacological analysis of atypical β -adrenoceptors in the guinea pig gastric fundus using the β_3 -adrenoceptor antagonist bupranolol. *Pharmacol.* **59**: 290–297.
- Kaumann, A.J. (1989). Is there a third heart β -adrenoceptor? *Trends Pharmacol. Sci.* **10**: 316–320.
- Koike, K., Takayanagi, I., Muramatsu, M., Ohki, S. and Horinouchi, T. (1994). Involvement of β_3 -adrenoceptor in the relaxation response in guinea pig taenia caecum. *Jpn. J. Pharmacol.* **66**: 213–220.
- Koike, K., Horinouchi, T. and Takayanagi, I. (1995a). Signal transduction pathway involved in β_3 -adrenoceptor-mediated relaxation in guinea pig taenia caecum. *Jpn. J. Pharmacol.* **68**: 41–46.
- Koike, K., Horinouchi, T. and Takayanagi, I. (1995b). Possible mechanisms of β -adrenoceptor-mediated relaxation induced by noradrenaline in guinea pig taenia caecum. *Eur. J. Pharmacol.* **279**: 159–

163.

- Koike, K., Ichino, T., Horinouchi, T. and Takayanagi, I. (1997). The β_2 - and β_3 -adrenoceptor-mediated relaxation induced by isoprenaline and salbutamol in guinea pig taenia caecum. *J. Smooth Muscle Res.* **33**: 99–106.
- Koike, K., Horinouchi, T. and Yamamoto, Y. (2000). The β_3 -adrenoceptor-mediated relaxation induced by epinephrine in guinea pig taenia caecum. *J. Smooth Muscle Res.* **36**: 93–99.
- Langin, D., Portillo, M.P., Saulnier-Blache, J.S. and Lafontan, M. (1991). Coexistence of three β -adrenoceptor subtypes in white fat cells of various mammalian species. *Eur. J. Pharmacol.* **199**: 291–301.
- Strosberg, A.D. (1997). Structure and function of the β_3 -adrenergic receptors. *Annu. Rev. Pharmacol. Toxicol.* **37**: 421–450.
- Tallarida, R. J., Cowan, A. and Adler, M.W. (1979). pA_2 and receptor differentiation: a statistical analysis of competitive antagonism. *Life Sci.* **25**: 637–654.
- Van Rossum, J.M. (1963). Cumulative dose-response curves, II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. *Arch. Int. Pharmacodyn.* **143**: 299–330.

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