The β3-Adrenoceptor-Mediated Relaxation Induced by Dopamine in Guinea Pig Taenia Caecum

Yurie AKIMOTO1, Takahiro HORINOUCHI1, Yoshio TANAKA1 and Katsuo KOIKE1

1Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences, 2-2-1, Miyama, Funabashi, Chiba 274-8510, Japan

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 pA₂ 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 β3-adrenoceptors.

Key words: β3-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 (β₁, β₂ and β₃) have been characterized by pharmacological, biochemical and molecular biological cloning approaches, and at present for each subtype (β₁, β₂ and β₃), the structure has been identified in turn (Strosberg, 1997). A large body of evidence indicates that the β₂-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 β₂- and β₃-adrenoceptors are involved in the β-adrenoceptor-mediated relaxation of the guinea pig taenia caecum (Koike et al., 1994; 1995a; 1995b), although the β₁-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 arylethanolamine class, are mediated by both the β₂- and the β₃-adrenoceptors in the guinea pig.
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 β3-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 β3-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 β1-adrenoceptors (Hoffman, 2001). However, it is not known whether the β3-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 ± 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.
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, Natik, 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 pD₂ 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 pD₂ values and intrinsic activities of these drugs are summarized in Table 1.

Propranolol (10⁻⁸–10⁻⁵ M), a nonselective β₁- and β₂-adrenoceptor antagonist, did not significantly affect the relaxant response to dopamine (Fig. 2). Moreover, phentolamine (10⁻⁸–10⁻⁵ M), a nonselective α₁- and α₂-adrenoceptor antagonist, did not significantly affect the relaxant response to dopamine (data not shown). Atenolol (3 × 10⁻⁷–3 × 10⁻⁴ M, a selective β₁-adrenoceptor antagonist), butoxamine (10⁻⁷–10⁻⁴ M, a selective β₂-adrenoceptor antagonist), prazosin (10⁻⁸–10⁻⁵ M, a selective α₁-adrenoceptor antagonist), yohimbine (10⁻⁸–10⁻⁵ M, a
selective α2-adrenoceptor antagonist), SCH 23390 (10−8 – 10−5 M, a selective D1-dopamine receptor antagonist) and haloperidol (10−8 – 10−5 M, a selective D2-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 pA2 value of 5.55 ± 0.10 and the slope of the regression line (1.13 ± 0.11) was not significantly different from unity. The pA2 values and Schild slopes for bupranolol against noradrenaline, adrenaline and dopamine are summarized in Table 2.

**Discussion**

Previously, we have demonstrated that both the β2- and the β3-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

---

**Table 1** The pD2 values and intrinsic activities of catecholamines

<table>
<thead>
<tr>
<th>Substance</th>
<th>pD2 value ± S.E.M.</th>
<th>I.A. ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Isoprenaline</td>
<td>8.35 ± 0.10</td>
<td>1.00</td>
</tr>
<tr>
<td>Adrenaline</td>
<td>7.36 ± 0.06</td>
<td>0.98 ± 0.05</td>
</tr>
<tr>
<td>Noradrenaline</td>
<td>6.61 ± 0.09</td>
<td>0.97 ± 0.06</td>
</tr>
<tr>
<td>Dopamine</td>
<td>4.52 ± 0.10</td>
<td>0.94 ± 0.04</td>
</tr>
</tbody>
</table>

Each value represents the mean ± 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 ± S.E.M. of six experiments.
adrenaline were resistant to the classical $\alpha$-adrenoceptor antagonist phentolamine and the classical $\beta$-adrenoceptor antagonist propranolol. Moreover, the selective $\alpha_1$, $\alpha_2$, $\beta_1$ and $\beta_2$-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 $\alpha$- and $\beta$-adrenoceptors, and dopamine receptors.

Bupranolol is reported to be a putative probe for the presence of $\beta_3$-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 $\beta_1$- and $\beta_2$-adrenoceptor antagonist and that, in high concentration (µM), bupranolol has the characterization of a $\beta_3$-adrenoceptor antagonist in addition to that of a non-selective $\beta_1$- and $\beta_2$-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

**Fig. 3.** Determination of the pA₂ value for bupranol against dopamine in the guinea pig taenia caecum. A: Antagonism of dopamine-induced relaxation by bupranolol. Control ( ), bupranolol $3 \times 10^{-6}$ M ( ), $10^{-5}$ M ( ), $3 \times 10^{-5}$ M ( ). Ordinate: relaxation (%), expressed as percentage of the maximum relaxation induced by isoprenaline, and abscissa: concentration (M) of dopamine. Each point represents the mean ± 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>
<thead>
<tr>
<th></th>
<th>Noradrenaline</th>
<th>Adrenaline</th>
<th>Dopamine</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bupranol</td>
<td>5.53 ± 0.06¹</td>
<td>5.87 ± 0.04²</td>
<td>5.55 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>(0.94 ± 0.09)</td>
<td>(1.10 ± 0.05)</td>
<td>(1.13 ± 0.11)</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.E.M. of six experiments. ¹Values from Koike et al., 1995. ²Values from Koike et al., 2000.
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 β₃-adrenoceptors.

Previous study has suggested that the relaxant responses to isoprenaline, a non-selective β₁- and β₂-adrenoceptor agonist, salbutamol and fenoterol, selective β₂-adrenoceptor agonists, which are the synthesized chemicals, in the guinea pig taenia caecum are mediated by both the β₂- and the β₃-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 β₁-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 β₁-adrenoceptors. These results suggest that the chemically synthesized β₁-adrenoceptor agonists recognize both the β₂- and the β₃-adrenoceptors, but that the adrenergic neurotransmitters may behave as a selective β₃-adrenoceptor agonist.

In conclusion, our results suggest that the relaxant response to dopamine in the guinea pig taenia caecum is mainly mediated by the β₃-adrenoceptors.

References


(Received April 10, 2003; Accepted May 9, 2003)