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Short communication

Pharmacological characterization of α_1-adrenoceptor in mouse iliac artery

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Abstract

Subtypes of α_1-adrenoceptor-mediated contraction to noradrenaline in the mouse iliac artery were determined (pharmaco-mechanically). Prazosin, 2-[2,6-dimethoxyphenoxyethyl]aminomethyl-1,4-benzodioxane hydrochloride (WB 4101) and 5-methylurapidil shifted the concentration–response curve for noradrenaline to the right, giving the pA_2 values of 9.30, 9.55 and 8.71, respectively. 8-[2-[4-(2-Methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378) shifted the concentration–response curve for noradrenaline to the right and the pA_2 value was 6.62. These results indicate that the contractile response to noradrenaline in the mouse iliac artery is predominantly mediated by the α_{1A} -adrenoceptor subtype.

Keywords: α_1-Adrenoceptor subtype; Iliac artery; Mouse; Noradrenaline

1. Introduction

α_1-Adrenoceptors consist of a heterogeneous family of receptors belonging to the superfamily of G-protein coupled receptors. The α_1-adrenoceptor classification comprises three native subtypes, termed α_{1A}, α_{1B} and α_{1D}, and their cloned counterparts are now designated α_{1a}, α_{1b} and α_{1d} (Hieble et al., 1995; Bylund et al., 1998). Muramatsu et al. (1990, 1995) proposed that the α_1-adrenoceptors can be pharmacologically divided into α_{1H} and α_{1L} subtypes with high ($pA_2>9$) and low ($pA_2<9$) affinity for prazosin, respectively. The α_{1A}-, α_{1B}- and α_{1D}-adrenoceptor subtypes are all included into the α_{1H}-adrenoceptor subtypes. The distribution of the various α_1-adrenoceptor subtypes is not homogeneous among the various vascular beds of different animal species (Guimaraes and Moura, 2001). Recently, targeted gene disruption has been increasingly used to elucidate the in vivo functions of several receptors, including some adrenoceptor subtypes (Link et al., 1995; Susulic et al., 1995; MacMillan et al., 1996; Rohrer et al., 1996; Cavalli et al., 1997). The potential functional changes occurring in knockout mice might allow, on one hand, to assign distinct functions to the receptor that has been deleted, and on the other, to test the functional redundancy among receptor subtypes. However, there is little information about the distribution of the α_1-adrenoceptor subtype in the normal mouse. Previously, we reported that (1) the α_{1D}-adrenoceptor subtype predominates in the mouse thoracic aorta (Yamamoto and Koike, 2001a); (2) the α_{1D}-like adrenoceptor subtype is present in the mesenteric artery; (3) the α_{1D}- and α_{1A}-adrenoceptor subtypes participate functionally to the contraction of mouse upper and lower abdominal aortas, respectively (Yamamoto and Koike, 2001b). In the present study, we tried to investigate the pharmacological characterization of α_1-adrenoceptor subtypes' mediated contraction in the mouse iliac artery.

2. Materials and methods

2.1. Mechanical responses

Male albino *ddY* mice (20–30 g) were killed by a blow on the head and the iliac artery was isolated and dissected free of excess fat and connective tissues. The intimal surface of the iliac artery was gently rubbed with a polyethylene tube to remove the endothelium. Functional loss of endothelial cells was confirmed by the loss of the relaxation response to acetylcholine (1 μM) of the tissue previously contracted by noradrenaline (100 μM). The iliac artery was cut into 4-mm ring segments. Each ring segment was suspended in a 20-ml organ bath filled with a Ringer–Locke solution (154 mM NaCl, 5.6 mM KCl, 2.2 mM $CaCl_2$, 2.1 mM $MgCl_2$, 5.9 mM

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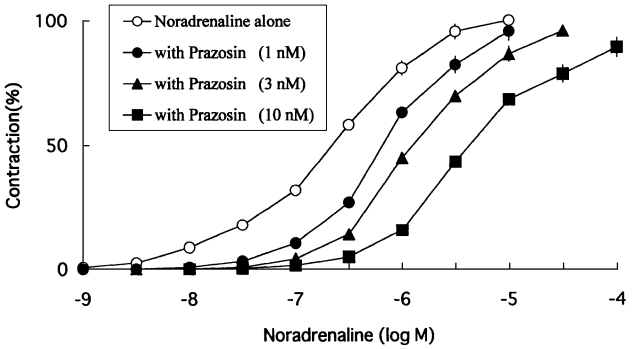

Fig. 1. Effects of prazosin on noradrenaline-induced contraction in the mouse iliac artery ($n = 7$). Ordinate: contraction (%), expressed as a percentage of the maximum contraction induced by noradrenaline (10 μM). Given are means ± S.E.M.

$NaHCO_3$ and 2.8 mM glucose) kept at 37 °C and bubbled with a mixture of 95% O_2 and 5% CO_2. The resting tension was 0.3 g. Tension was monitored continuously and recorded isometrically by a force-displacement transducer. Experiments were conducted in the presence of propranolol (10 μM), yohimbine (300 nM), desmethylimipramine (100 nM) and normetanephrine (1 μM) to block β-adrenoceptors and α_2-adrenoceptors and to inhibit neural and non-neural uptake of noradrenaline, respectively. The strips were allowed to equilibrate for 90 min, were then contracted with noradrenaline (100 nM) and allowed to equilibrate for 30 min after washout. This was repeated until two successive contractions of approximately equal sizes had been obtained. The competitive antagonistic activities were expressed as pA_2 values (negative logarithms of dissociation constant). The concentration–response curves for the agonist were obtained cumulatively. Contraction was expressed as a percentage of the maximal response produced by the agonist. After determination of control concentration–response curves, the strips were equilibrated with a competitive antagonist for 15 min. Concentration–response curves were then obtained in the presence of three different concentrations of the antagonist and the procedure was repeated with a high (either 3- or 10-fold increase) concentration of the antagonist in the same preparation. In the present study, prazosin (1, 3 and 10 nM), 2-[2,6-dimethoxyphenoxyethyl]aminomethyl-1,4-benzodioxane hydrochloride (WB 4101) (1, 3 and 10 nM), 5-methylurapidil (10, 30 and 100 nM) and 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378) (1, 3 and 10 μM) were used to determine the pA_2 values. After determination of the control concentration–response curves, two or three successive cumulative concentration–response curves for the agonist were determined. The curves were nearly superimposable and changes in insensitivity, sensitization, or desensitization were minimal (data not shown). The pA_2 values were calculated according to a method (Tallarida et al., 1979) which was originally described by Arunlakshana and Schild (1959). Antagonist potency was expressed as the dissociation constant K_B is the equation KB=[B]/(DR − 1), where [B] is the concentration of antagonist and DR is the agonist dose ratio produced by the antagonist as compared to control. Antagonist pA_2 values were obtained from the *X*-intercept of plot of log (agonist DR − 1) against log antagonist concentration using regression analysis.

2.2. Data analysis

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

2.3. Drugs

The following drugs were used: (−)-noradrenaline bitartrate (Wako-Junyaku, Osaka, Japan); 8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]-ethyl]-8-azaspiro[4,5]decane-7,9-dione dihydrochloride (BMY 7378), 5-methylurapidil and 2-[2,6-dimethoxyphenoxyethyl]aminomethyl-1,4-benzodioxane hydrochloride (WB 4101) (Research Biochemicals, Natick, MA); desmethylimipramine hydrochloride, (±)-normetanephrine hydrochloride, (±)-propranolol hydrochloride, prazosin hydrochloride and yohimbine hydrochloride (Sigma, St. Louis, MO).

5-methylurapidil was dissolved in dimethylsulfoxide (DMSO) at the initial concentration of 2 mM and diluted in distilled water. All other drugs were dissolved in distilled water.

3. Results

In the endothelium-denuded mouse iliac artery, noradrenaline evoked contraction in a concentration-dependent manner. The pD_2 value of noradrenaline is 6.61 ± 0.08. The contractile response to noradrenaline was antagonized by prazosin in a concentration-dependent manner. Prazosin caused parallel shifts to the right of the concentration–response curve for noradrenaline without affecting the maximum responses (Fig. 1). Schild regression analysis carried out for prazosin against noradrenaline gave the pA_2 value of 9.30 (Table 1). The slope of the Schild regression line was not significantly different from unity (Table 1). WB 4101, 5-methylurapidil and BMY 7378 also caused parallel shifts to

Table 1
The pA_2 values of α_1-adrenoceptor antagonists against noradrenaline in the mouse iliac artery

Antagonist	pA_2 value	Slope	*n*
Prazosin	9.30 ± 0.06	0.97 ± 0.06	7
WB 4101	9.55 ± 0.05	1.03 ± 0.02	8
5-Methylurapidil	8.71 ± 0.09	1.11 ± 0.06	10
BMY 7378	6.62 ± 0.09	1.10 ± 0.04	5

Each value indicates the mean ± S.E.M.

the right of the concentration–response curve for noradrenaline without affecting the maximum responses. Schild regression analysis carried out for WB 4101, 5-methylurapidil and BMY 7378 against noradrenaline gave the pA_2 values of 9.55, 8.71 and 6.62, respectively (Table 1). The slopes of the Schild regression lines were not significantly different from unity (Table 1).

4. Discussion

In the present study, prazosin shifted the concentration–response curve for noradrenaline to the right without affecting the maximum responses. The Schild slope of 0.97 indicates the competitive nature of the antagonism. The pA_2 value of 9.30 (pA_2>9) is in agreement with receptors at which prazosin shows high affinity, ruling out the presence of the α_{1L} subtypes (Flavahan and Vanhoutte, 1986; Muramatsu et al., 1990). The concentration–response curves for noradrenaline were shifted to the right by the α_{1A}-adrenoceptor-selective antagonists, WB 4101 or 5-methylurapidil. Neither slope of the Schild regression lines was significantly different from unity, suggesting a simple competitive antagonism (Table 1). The pA_2 values of 9.55 and 8.71 for WB 4101 and 5-methylurapidil, respectively, observed in this study are in agreement with the reported affinity values for these drugs at the α_{1A}-adrenoceptor subtype (Honner and Docherty, 1999). Moreover, the concentration–response curve for noradrenaline was rightward shifted by the 1–10 μM BMY 7378, and the pA_2 value was 6.62, suggesting the contraction by noradrenaline mediated through the non-α_{1D}-adrenoceptor subtype (Honner and Docherty, 1999; Jarajapu et al., 2001). The pA_2 value for BMY 7378 is in agreement with the reported value at the α_{1A}-adrenoceptor subtype (Honner and Docherty, 1999). Goetz et al. (1995) showed that pK_B values of BMY 7378 for α_{1a}, α_{1b} and α_{1d} subtypes expressed in rat fibroblasts were 6.6, 7.2 and 9.4, respectively. Furthermore, the pA_2 values of 8.3 and 7.1 for WB 4101 and 5-methylurapidil, respectively, were published for the well-known values at the α_{1B}-adrenoceptor subtype (Honner and Docherty, 1999). Therefore, the predominant involvement of the α_{1B}-adrenoceptor subtype in noradrenaline-mediated contractile responses may be ruled out.

In conclusion, the present study indicates that the contractile response to noradrenaline in the mouse iliac artery is predominantly mediated by the α_{1A}-adrenoceptor subtype.

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