Differences of Antagonism for a Selective $\alpha_{1D}$-Adrenoceptor Antagonist BMY 7378 in the Rabbit Thoracic Aorta and Iliac Artery

Mitsutoshi SATOH, Keisuke ENOMOTO, Issei TAKAYANAGI and Katsuo KOIKE
Department of Chemical Pharmacology, Toho University School of Pharmaceutical Sciences

Abstract

Based on the affinity of $\alpha_{1D}$-adrenoceptor subtype for a selective antagonist BMY 7378, we studied its functional role in rabbit thoracic aorta and iliac artery, and evaluated the subtypes of the $\alpha_1$-adrenoceptors that are activated by phenylephrine (a full agonist) and tizanidine (a partial agonist). In thoracic aorta, the concentration-response curves of phenylephrine and tizanidine were antagonized by BMY 7378 with low potency ($pA_2$ values $6.68\pm0.06$ and $6.67\pm0.06$, slopes of Schild plot $1.06\pm0.04$ and $1.01\pm0.04$, respectively). On the other hand, in iliac artery concentration-response curves for phenylephrine were potently antagonized by a low concentration of BMY 7378, and the slope ($0.75\pm0.02$) of the Schild plot was significantly different from unity. In iliac artery, a concentration-response curve of tizanidine was antagonized by BMY 7378 with low potency ($pA_2$ value $6.64\pm0.08$, slope of Schild plot $1.01\pm0.05$). These results suggest that an $\alpha_{1D}$-adrenoceptor subtype contributes to $\alpha_1$-adrenoceptor mediating muscle contraction in iliac artery, but not in thoracic aorta of rabbit, and that it is activated by a full agonist phenylephrine but not by a partial agonist tizanidine.

Key Words: $\alpha_1$-adrenoceptors, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane 7,9-dione dihydrochloride, thoracic aorta, iliac artery, rabbit

Introduction

$\alpha_1$-Adrenoceptors are pharmacologically divided into three major subtypes, $\alpha_{1A}$-, $\alpha_{1B}$- and $\alpha_{1D}$-adrenoceptor subtypes. The $\alpha_{1D}$-adrenoceptor subtype has a high affinity for the antagonist, BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decane 7,9-dione dihydrochloride (Saussy et al., 1994), which is a useful pharmacological probe for studying $\alpha_{1D}$-adrenoceptor function. BMY47378 possessed approximately more than 10 fold higher affinity ($pK_i=8.2\sim9.4$) for the $\alpha_{1D}$-adrenoceptor subtype than those ($pK_i=6.1\sim6.6$ and $pK_i=6.7\sim7.2$, respectively) for the $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes in cells expressing cloned human or nonhuman and potent antagonistic effects ($pA_2=8.9$ or $pK_b=8.3$) in rat
thoracic aorta existing native $\alpha_{1D}$-adrenoceptor subtypes (Goetz et al., 1995; Kenny et al., 1995). Recently a number of investigators have reported that the $\alpha_{1D}$-adrenoceptor subtype exists in various tissues (Wada et al., 1996; Nasu et al., 1998; Price et al., 1994; Scofield et al., 1995) and also plays an important role in muscle contraction in rat aorta using BMY 7378 (Kenny et al., 1995; Testa et al., 1995), but the characteristics of $\alpha_{1D}$-adrenoceptor subtype have not heretofore been studied using BMY 7378 in aorta and arteries of rabbit. Previously, Takayanagi et al. (1991) showed that the thoracic aorta and iliac artery of rabbit contain both $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes and demonstrated the effects of full and partial agonists for these subtypes. Recently, Satoh et al. (1998) noted the possibility of an $\alpha_{1D}$-adrenoceptor subtype co-existing in renal and iliac artery of rabbit. To determine the existence of an $\alpha_{1D}$-adrenoceptor subtype and to clarify the effects of the full and partial agonists on $\alpha_{1D}$-adrenoceptor subtypes in rabbit vessels, we studied pharmacologically the contractile responses for phenylephrine and tizanidine in the rabbit thoracic aorta and iliac artery, taking heterogeneity in $\alpha_1$-adrenoceptors into consideration.

**Methods**

**Mechanical responses**

Male albino rabbits weighing 2.0–3.0 kg were anesthetized with an intravenous injection of pentobarbital sodium (50 mg/kg) and killed by bleeding from the carotid arteries. Thoracic aorta and iliac arteries were quickly removed and dissected free of excess fat and connective tissue in oxygenated physiological saline solution (PSS) of the following composition (in millimoles): NaCl, 118; MgCl₂, 1.2; CaCl₂, 2.5; KH₂PO₄, 1.2; NaHCO₃, 25 and glucose, 11.0 dissolved in distilled water (pH = 7.4 at 37°C). The solution contained propranolol (10⁻⁶ M), yohimbine (3 × 10⁻⁷ M), desmethylimipramine (10⁻⁷ M), and normetanephrine (10⁻⁶ M) to block $\beta$-adrenoceptors and $\alpha_2$-adrenoceptors and to inhibit neural and non-neural uptake of catecholamines, respectively. The arteries were cut into helical strips about 10 mm in length and 2 mm in width. In order to avoid the possible involvement of endothelium-derived relaxing factor in the mechanical response, the endothelial cells were removed by gently rubbing with a cotton probe, and the functional loss of endothelial cells was confirmed by the loss of the relaxation response to acetylcholine (10⁻⁶ M) in phenylephrine-precontracted aorta and artery. The strips were suspended in a 20-ml organ bath filled with PSS gassed with a mixture of 95% O₂ and 5% CO₂ and maintained at 37°C. The response to an agonist was isometrically recorded under a resting tension of 1 g for thoracic strips, 0.5 g for iliac arteries. The strips were allowed to equilibrate for 90 min. They were contracted with phenylephrine (10⁻⁶ M) and allowed to equilibrate for 30 min after washout. This was repeated until two successive contractions of approximately equal size had been obtained. After determination of control concentration–response curves obtained from cumulative application of agonist, the strips were equilibrated with a competitive antagonist for 10 min. Concentration–response curves were then obtained in the presence of the antagonist and the procedure was repeated with a high (either 3- or 10-fold) concentration of the antagonist in the same preparation. The curves were nearly superimposable and changes in sensitivity, sensitization, or desensitization were
α₁D-Adrenoceptors of aorta and artery

minimal. The competitive antagonistic activities were expressed as pA₂ values (negative logarithms of the dissociation constant). The pA₂ values were calculated according to the method of Arunlakshana and Schild (1959).

Statistics
Numerical results are expressed as means ± S.E., and statistical significance was calculated with Student's t-test. A P value less than 0.05 was considered to indicate a significant difference.

Drugs
The following drugs were used: BMY 7378 (8-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-8-azaspiro[4,5]decan-7,9-dione dihydrochloride (Research Biochemicals Inc., Natick, MA); tizanidine hydrochloride (Sandoz, Tokyo, Japan); phenylephrine hydrochloride, desmethylimipramine hydrochloride, (+)-normetanephrine hydrochloride, (+)-propranolol hydrochloride, and yohimbine hydrochloride (Sigma, St. Louis, USA), all in powder form. Pentobarbital sodium (Abbott Lab., North Chicago, IL, USA). Other chemicals used were of analytical grade.

Results
Phenylephrine produced concentration-dependent contractions of thoracic aorta and iliac artery with pD₂ values of 6.63 ± 0.06 (n = 4) and 6.75 ± 0.10 (n = 4), respectively. Tizanidine also produced concentration-dependent contractions of thoracic aorta and iliac artery with pD₂ values of 5.12 ± 0.02 (n = 4) and 5.41 ± 0.04 (n = 4), respectively. The maximum amplitude of tizanidine-produced contractions of thoracic aorta and iliac artery was 52.9 ± 2.25 (n = 4) and 71.1 ± 2.6 (n = 4), respectively. Selective α₁D-adrenococeptor subtype antagonist BMY 7378 shifted the concentration-response curves for phenylephrine to the right in these vessels (Figs. 1A and B). In thoracic aorta, the Schild regression obtained from the results of antagonism between BMY 7378 (300-3,000 nM) and phenylephrine yielded a straight line with a slope of unity (1.06 ± 0.04), suggesting a simple competitive antagonism, and the pA₂ value for BMY 7378 against phenylephrine was 6.68 ± 0.06. However, in iliac artery Schild plot of the results obtained from the inhibition by BMY 7378 (1-30 nM) for phenylephrine yielded a slope significantly different from unity (0.75 ± 0.02), suggesting that phenylephrine acted through at least two receptor populations. In both thoracic aorta and iliac artery (Figs. 2A and B), the Schild regression obtained from the results of antagonism between BMY 7378 and tizanidine yielded straight lines with slopes of unity (1.01 ± 0.04 and 1.01 ± 0.05), suggesting a simple competitive antagonism, and pA₂ values for BMY 7378 (100-1,000 nM) against tizanidine were 6.67 ± 0.06 and 6.64 ± 0.08, respectively.

Discussion
BMY 7378 is higher affinity for cloned human α₁D-adrenococeptor subtypes (pKᵢ = 8.2−9.4)
Fig. 1 Effects of BMY 7378 on phenylephrine-induced contraction of rabbit (A) thoracic aorta and (B) iliac artery. Ordinate: Contraction (%) which is expressed as a percentage of the contractile response to phenylephrine ($10^{-5}$ M). Abscissa: logarithm of phenylephrine concentration (M). •, phenylephrine alone; ○, $10^{-9}$ M BMY 7378; ▲, $3 \times 10^{-9}$ M BMY 7378; △, $10^{-8}$ M BMY 7378; ■, $3 \times 10^{-8}$ M BMY 7378; ●, $3 \times 10^{-7}$ M BMY 7378; ○, $10^{-6}$ M BMY 7378; ▼, $3 \times 10^{-6}$ M BMY 7378. Inset, Schild plots. Ordinate: logarithm of equieffective concentration ratio (CR) of phenylephrine minus 1. Abscissa: logarithm of molar concentration of BMY 7378. Each value is presented as the mean ± S.E. (bar) of four experiments.

than that for cloned human $\alpha_{1a}$- (pKᵢ=6.1~6.2) or $\alpha_{1b}$-adrenoceptor subtypes (pKᵢ=6.2~7.2), and is also higher affinity for cloned rat $\alpha_{1d}$-adrenoceptor subtypes (pKᵢ=8.2) and native rat $\alpha_{1b}$-adrenoceptor subtypes (pKᵢ=8.3 and pA₂=8.7~8.9) than that for cloned bovine $\alpha_{1a}$- (pKᵢ=6.1) or hamster $\alpha_{1b}$-adrenoceptor subtypes (pKᵢ=6.2) (Goetz et al., 1995; Kenny et al., 1995; Lachnit et al., 1997). Based on the property of the selective $\alpha_{1b}$-antagonist BMY 7378, in rabbit iliac artery we revealed a co-existence of $\alpha_{1d}$-adrenoceptor subtype in addition to $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes. As shown in Figure 1B, in iliac artery the concentration-response curve of phenylephrine was potently antagonized by a low concentration of BMY 7378,
Fig. 2  Effects of BMY 7378 on tizanidine–induced contraction of rabbit (A) thoracic aorta and (B) iliac artery. Ordinate: Contraction (%) which is expressed as a percentage of the contractile response to phenylephrine (10^{-6} M). Abscissa: logarithm of tizanidine concentration (M). ●, tizanidine alone; □, 10^{-7} M BMY 7378; ●, 3 × 10^{-7} M BMY 7378; ○, 10^{-6} M BMY 7378. Inset, Schild plots. Ordinate: logarithm of equieffective concentration ratio (CR) of tizanidine minus 1. Abscissa: logarithm of molar concentration of BMY 7378. Each value is presented as the mean±S.E. (bar) of four experiments.
that the $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes contribute to the norepinephrine-induced contraction in rabbit thoracic aorta. It is reported that BMY 7378 was a selective $\alpha_{1D}$-adrenoceptor antagonist and possessed an affinity more than approximately 100 fold higher for the $\alpha_{1D}$-adrenoceptor subtype than those for the $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes (Goetz et al., 1995; Kenny et al., 1995; Lachnit et al., 1997). As shown in Figure 1B, in iliac artery, the intercept of the Schild plot was approximately 8.5 which is similar to the value ($pK_B 8.3$) for the $\alpha_{1D}$-adrenoceptor subtype which was reported by Kenny et al. (1995). On the other hand, the concentration-response curves of tizanidine were shifted in a parallel manner by BMY 7378 with low potency in both thoracic aorta and iliac artery. Schild plots of the results obtained from the inhibition by BMY 7378 yielded straight lines with slopes of unity (Table 1), suggesting a simple competitive antagonism, and the $pA_2$ values for BMY 7378 against tizanidine were $6.67 \pm 0.06$ and $6.64 \pm 0.08$, respectively. These observations are similar to that obtained from antagonism between phenylephrine and BMY 7378 in thoracic aorta (Fig. 1A and Table 1). These $pA_2$ values are significantly different from that for the $\alpha_{1D}$-adrenoceptor subtype (Kenny et al., 1995; Testa et al., 1995; Eltze, 1997), and also similar to those for $\alpha_{1A}$ and $\alpha_{1B}$. These findings suggest that tizanidine-induced contractile responses are not due to the activation of $\alpha_{1D}$-adrenoceptor subtypes. And in a previous report Takayanagi et al. (1991) suggested that in thoracic aorta and iliac artery a full agonist such as phenylephrine produced a muscle contraction through both $\alpha_{1A}$- and $\alpha_{1B}$-adrenoceptor subtypes, whereas a partial agonist such as tizanidine produced one only through the $\alpha_{1A}$-adrenoceptor subtype. Satoh et al. (1992b) also reported a similar observation in rabbit thoracic aorta using a full agonist norepinephrine and a partial agonist clonidine. Taken together, it is suggested that a full agonist, such as phenylephrine, induces contraction through $\alpha_{1A}$-, $\alpha_{1B}$-, and $\alpha_{1D}$-adrenoceptor subtypes and a partial agonist, such as tizanidine induces one only through $\alpha_{1A}$-adrenoceptor subtype.

In conclusion, the present functional study using BMY 7378 has demonstrated that in rabbit iliac artery the $\alpha_1$-adrenoceptors that mediate contractions include pharmacological characteristics of the $\alpha_{1D}$-adrenoceptor subtype as well, but rabbit thoracic aorta at least does not have a functional $\alpha_{1D}$-adrenoceptor subtype. Also the $\alpha_{1D}$-adrenoceptor subtype is activated by a full agonist, phenylephrine, but not by a partial agonist, tizanidine.

---

### Table 1

|               | n  | $pA_2$ Value | Slope     |
|---------------|----|--------------|-----------|
| **Thoracic aorta** |    |              |           |
| phenylephrine | 4  | $6.68 \pm 0.06$ | $1.06 \pm 0.04$ |
| tizanidine    | 4  | $6.67 \pm 0.06$ | $1.01 \pm 0.04$ |
| **Iliac artery** |    |              |           |
| phenylephrine | 4  |              | $0.75 \pm 0.02^*$ |
| tizanidine    | 4  | $6.64 \pm 0.08$ | $1.01 \pm 0.05$ |

Each value is presented as a mean±S.E. of four separate experiments.

*Significant difference from unity ($P<0.05$).
Acknowledgment

This study was supported by a grant-in-aid for Scientific Research from the Ministry of Education, Science and Culture, Japan (No. 09772003).

References

Arunlakshana, O. and Schild, H.O. (1959) Some quantitative uses of drug antagonists. Br. J. Pharmacol., 14: 48-59.

Eltze, M. (1997) Affinity of the miotic drug, dapiprazole, at α1-adrenoceptor subtypes A, B and D. J. Pharm. Pharmacol., 49: 1091-1095.

Goetz, A.S., King, H.K., Ward, S.D., True, T.A., Rimele, T.J. and Saussy, D.L. Jr. (1995) BMY 7378 is a selective antagonist of the D subtype of α1-adrenoceptors. Eur. J. Pharmacol., 272: R5-6.

Kenny, B.A., Chalmers, D.H., Philpott, P.C. and Naylor, A.M. (1995) Characterization of an α1D-adrenoceptor mediating the contractile response of rat aorta to noradrenaline. Br. J. Pharmacol., 115: 981-986.

Lachnit, W.G., Tran, A.M., Clarke, D.E. and Ford, A.P. (1997) Pharmacological characterization of α1A-adrenoceptor mediating contractile responses to noradrenaline in isolated caudal artery of rat. Br. J. Pharmacol., 120: 819-826.

Nasu, K., Moriyama, N., Fukasawa, R., Tsujimoto, G., Tanaka, T., Yano, J. and Kawabe, K. (1998) Quantification and distribution of α1-adrenoceptor subtype mRNAs in human proximal urethra. Br. J. Pharmacol., 123: 67-73.

Scofield, M.A., Liu, F., Abel, P.W. and Jeffries, W.B. (1995) Quantification of steady state expression of mRNA for alpha-1 adrenergic receptor subtypes using reverse transcription and a competitive polymerase chain reaction. J. Pharmacol. Exp. Ther., 275: 1035-1042.

Takayanagi, I., Harada, M., Koike, K. and Satoh, M. (1991) Differences in α1-adrenoceptor mechanisms for phenylephrine and tizanidine in rabbit thoracic aorta and common iliac artery. Can. J. Physiol. Pharmacol., 69: 1819-1824.

Testa, R., Destefani, C., Guarneri, L., Poggesi, E., Simonazzi, I., Taddei, C. and Leonardi, A. (1995) The α1D-adrenoceptor subtype is involved in the noradrenaline-induced contractions of rat aorta. Life Sci., 57: PL159-163.
Wada, T., Otsu, T., Hasegawa, Y., Mizuchi, A. and Ono, H. (1996) Characterization of alpha 1-adrenoceptor subtypes in rat spinal cord. *Eur. J. Pharmacol.*, **312**: 263-266.

(Received November 9, 1998: Accepted December 7, 1998)