Existence of Functional $\alpha_{1A-}$ and $\alpha_{1D-}$ but No $\alpha_{1B-}$ Adrenoceptor Subtypes in Rat Common Carotid Arteries

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ABSTRACT—Using the cannula inserting method, vasoconstrictor responses to $\alpha_1$-adrenoceptor agonists (noradrenaline [NA], phenylephrine [PE] and methoxamine [ME]) and effects of $\alpha_1$-adrenoceptor antagonists (WB4101, chloroethylclonidine [CEC] and BMY7378) were investigated in isolated and perfused rat common carotid arteries. The rank order of agonist potency and efficacy was NA = PE > ME. Either WB4101 or BMY7378 inhibited NA- and PE-induced constrictions in a dose-related manner. CEC did not inhibit the NA- and PE-induced responses. The ME-induced responses were also significantly blocked by either WB4101 or BMY7378. From these results, it is concluded that there are functional $\alpha_{1A-}$ and $\alpha_{1D-}$ adrenoceptor subtypes in rat common carotid arteries, but no functional $\alpha_{1B-}$ subtype.

Keywords: Cannula inserting method, Rat common carotid artery, $\alpha_1$-Adrenoceptor subtype

Previously, in isolated rat common carotid arteries we investigated pharmacological features of vascular responses (1), using the cannula insertion technique (2, 3). It was demonstrated that noradrenaline and phenylephrine caused a long-lasting vasoconstriction (30–60 min) even in a single injection of each compound, although other vasoconstrictor compounds (5-HT, PGF$_2\alpha$, angiotensin II or KCl) caused a relatively short-lasting constriction (within 20 min), indicating that this artery responded well to the $\alpha_1$-adrenoceptor agonist. On the other hand, selective $\alpha_2$-adrenoceptor agonists, clonidine and xylazine, did not cause any vascular response in the same arterial preparation, indicating that there are no functional $\alpha_2$-adrenoceptors (1). Thus, it is interesting to clarify the $\alpha_1$-adrenoceptor subtypes in the rat common carotid artery. $\alpha_1$-Adrenoceptors are a heterogeneous family of G-protein coupled receptors. It is recognized that there are three functional $\alpha_1$-adrenoceptor subtypes, $\alpha_{1A}$, $\alpha_{1B}$ and $\alpha_{1D}$, corresponding to the cloned subtypes, $\alpha_{1a}$, $\alpha_{1b}$ and $\alpha_{1d}$, respectively (4, 5). Recently, a selective $\alpha_{1D}$-adrenoceptor antagonist, BMY7378, was introduced (6). Therefore, in the present study we investigated the postsynaptic $\alpha_1$-adrenoceptor subtypes of the isolated rat common carotid artery by using selective $\alpha_{1A-}$, $\alpha_{1B-}$ and $\alpha_{1D-}$adrenoceptor subtype antagonists (WB4101, chloroethylclonidine and BMY7378, respectively).

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MATERIALS AND METHODS

Male Wistar rats (weighing an average of 220 g, aged 7 weeks) were used in this study. After ether anesthesia and treatment with sodium heparin (200 units/kg, i.v.), these animals were sacrificed by rapid exsanguination and then the common carotid artery was excised and immersed immediately in cold Ringer solution at 4°C. Isolated common carotid arteries that were 10 to 11 mm in length and 0.8 to 0.9 mm in outer diameter were selected for study. A stainless steel cannula with small holes 2 mm from the distal sealed end (25 gauge and 3 cm in length) was carefully inserted into each vessel segment to avoid injury of the intraluminal surface of the isolated vessel. Segments were set up in the bath for preparation as described by Tsuji and Chiba (3). The perfusion solution contained: 118 mmol/l NaCl, 4.7 mmol/l KCl, 2.5 mmol/l CaCl$_2$, 25 mmol/l NaHCO$_3$, 1.2 mmol/l MgSO$_4$, 1.2 mmol/l KH$_2$PO$_4$ and 11 mmol/l glucose, and it was bubbled with 95% O$_2$ and 5% CO$_2$, which maintained the pH of the solution at 7.2–7.4. The bath and perfusion circuit were warmed at 37°C with a thermostump (Model FE2; Haake, Karlsruhe, Germany). The speed of the perfusion pump was initially adjusted so that the perfusion pressure was maintained at 50–70 mmHg throughout the experiments. The average flow rate was 1.4 ml/min. The vasoconstriction was there-
chloride (Sumitomo Chemicals, Tokyo), noradrenaline hydrochloride (Sankyo Co., Tokyo), phenylephrine hydrochloride (Tokyo Kasei Co., Tokyo), methoxamine hydrochloride (Kowa Co., Tokyo), WB4101 (2(2,6-dimethoxyphenoxethyl)amino-methyl-1,4 benzodioxane hydrochloride) (Amersham, Little Chalfont, UK), chloroethylclonidine dihydrochloride (Funakoshi Co., Tokyo), BMY7378 (dihydrochloride (8-(2)-4-(2-methoxyphenyl)1-piperazinyl)ethyl)-8-azaspiro(4,5)-decane-7,9-dione dihydrochloride) (Research Biochemicals, Inc., Natick, MA, USA). All drugs were dissolved in distilled water, and diluted in physiological saline. The stock solutions were kept at \(-20^\circ C\) until used.

After the vessel preparation was set up and perfused about 60 min, propranolol solution (1 mmol/l) (which was diluted in physiological saline) was perfused for 30 min. Then the experiments were started.

The drugs, except for propranolol, were bolusly administered intraluminally. The drug solution was given into the rubber tube connecting the cannula in a volume of 10-20 µl by a microinjector (Terumo Co., Tokyo) for approximately 4 s. After the responses to the agonists finished and the perfusion pressure returned to the basal level, the next dose of drugs was administered.

The agonist-induced dose-response curves were examined after treatments with increasing concentrations of each antagonist. Usually one-agonist-antagonist relationship was obtained from one vessel preparation.

Vasoconstrictor responses are expressed as the maximal changes in perfusion pressure (mmHg) from their basal levels. The data are shown as the mean ± S.E.M. An analysis of variance with Bonferroni’s test was used for the statistical analysis of multiple comparisons of data. \(P\) values less than 0.05 were considered statistically significant.

RESULTS

Vascular responses to 3 different \(\alpha\)-adrenoceptor agonists, noradrenaline, phenylephrine and methoxamine

The control basal perfusion pressure was 62 ± 2 mmHg (n = 12). When noradrenaline was administrated into the cannulated and perfused rat common carotid artery, an immediate increase in perfusion pressure was obtained transiently, and repetitive administrations induced almost the same grade of vasoconstrictions under the conditions where the previous response disappeared completely. However, a relatively large amount of noradrenaline in doses more than \(10^{-8}\) mol usually caused a long-lasting vasoconstriction. The time at which the pressure returned to the control level was frequently over 60 min as reported before (1). Thus, it was difficult to use a dose of more than \(10^{-8}\) mol for the pharmacological analysis. Since \(10^{-7}\) mol noradrenaline caused almost the same increase in perfusion pressure as that at \(10^{-8}\) mol in 4 experiments (data not shown), we considered that the vasoconstrictor response to \(10^{-8}\) mol noradrenaline might be almost the maximum. The vasoconstrictor response to phenylephrine was almost the same as that to noradrenaline in a dose-related manner, but the duration was slightly shorter. When compared to the response to noradrenaline and phenylephrine, methoxamine-induced vasoconstriction was consistently less than a half of the noradrenaline- or phenylephrine-induced one. Summarized data are shown in Fig. 1.

Fig. 1. Vascular responses to \(\alpha\)-adrenoceptor agonists, increasing doses of noradrenaline, phenylephrine or methoxamine of isolated rat common carotid arteries. PP, perfusion pressure.
noradrenaline-induced response was almost completely inhibited.

**Effects of WB4101, chloroethylclonidine and BMY7378 on phenylephrine-induced constriction responses**

After treatment with WB4101, the phenylephrine-induced constrictions were inhibited significantly in a dose-related manner as shown in Fig. 3A. The responses were completely inhibited by 1 nmol WB4101.

After 100 nmol chloroethylclonidine, phenylephrine-induced responses were unmodified as shown in Fig. 3B.

After BMY7378, the phenylephrine-induced dose-response curve was inhibited in a dose-related manner as shown in Fig. 3C. A relatively large dose of 100 nmol BMY7378 completely inhibited the phenylephrine-induced responses.
Effects of WB4101, chloroethylclonidine and BMY7378 on methoxamine-induced vasoconstrictions

As mentioned above, methoxamine produced a relatively weak increase in perfusion pressure, showing a weak vasoconstriction. The vasoconstrictions in small doses of methoxamine were variable and sometimes undetectable. Therefore, the effects of each \( \alpha_1 \)-adrenoceptor subtype blocking agent were examined for 10\(^{-8} \) mol methoxamine alone. Thus, the blocking effects of antagonists are shown as \% changes.

After 0.01, 0.1 and 1 nmol WB4101, methoxamine-induced constrictions were markedly inhibited as shown in Fig. 4A.

After 1 and 10 nmol BMY7378, methoxamine-induced responses were also significantly inhibited as shown in Fig. 4B.

On the other hand, 100 nmol chloroethylclonidine was without effect on the response to methoxamine (four experiments).

DISCUSSION

In the past we studied the functional characteristics of vascular responses of isolated rat common carotid arteries (1, 7, 8). We found that a bolus administration of noradrenaline and phenylephrine caused a long-lasting vasoconstrictor response (1). In the present study, we examined effects of three different \( \alpha_1 \)-adrenoceptor agonists, noradrenaline, phenylephrine and methoxamine. When compared to noradrenaline and phenylephrine, methoxamine apparently showed lower potency and efficacy. It has been reported that methoxamine is a relatively selective \( \alpha_{1A} \)-adrenoceptor agonist (9), and Minneman et al. (10) reported that methoxamine is approximately 20-fold more potent to activate \( \alpha_{1A} \) than \( \alpha_{1B} \) and \( \alpha_{1D} \)-adrenoceptors in mediating \([^{3}H] \)inositol phosphate formation. It was suggested that \( \alpha_{1A} \)-adrenoceptors might not be so abundant in the rat mesenteric artery (10, 11). On the other hand, noradrenaline and phenylephrine show relative selectivity for the \( \alpha_{1D} \)-adrenoceptor subtype (10, 11). Hussain and Marshall (12, 13) reported that \( \alpha_1 \)-adrenoceptor in rat aortae, pulmonary and mesenteric arteries are primarily of the \( \alpha_{1D} \)-adrenoceptor subtype and possibly \( \alpha_{1B} \)-adrenoceptor subtype, using the agonist, phenylephrine, methoxamine; and an \( \alpha_{1D} \)-adrenoceptor agonist, P7480 (N-(4-pyridinyl)-1H-indol-1-amine) (14); and subtype-selective antagonists, WB4101, BMY7378, and others. De Oliveira et al. (15) reported in rat carotid arteries that the vasoconstriction caused by phenylephrine was inhibited by a selective \( \alpha_{1B} \)-adrenoceptor antagonist, chloroethylclonidine, in a concentration-dependent manner. They also reported that WB4101 very readily inhibited the phenylephrine-induced vasoconstriction, suggesting that there are functional \( \alpha_{1A} \) and \( \alpha_{1B} \)-adrenoceptors in the rat carotid arteries. However, de Oliveira et al. (15) showed that phenylephrine-induced vasoconstrictions were inhibited by WB4101 with IC\(_{50}\) values between 10\(^{-10} \) and 10\(^{-9} \) mol/l for the rat carotid artery, showing the abundant existence of the \( \alpha_{1A} \)-adrenoceptor subtype. On the other hand, they also showed that those were inhibited by an extremely high concentration of chloroethylclonidine with IC\(_{50}\) values at about 10\(^{-6} \) mol/l, indicating the existence of few receptors of the \( \alpha_{1B} \) subtype. Moreover, they did not use a selective \( \alpha_{1D} \)-adrenoceptor subtype antagonist. Thus, it seems that \( \alpha_{1B} \) adrenoceptors are functionally rather few in number, although de Oliveira et al. (15) reported the existence of \( \alpha_{1B} \) receptors on the rat carotid artery.

In the present study, each \( \alpha_1 \)-agonist-induced response was readily inhibited by WB4101, an \( \alpha_{1A} \)-adrenoceptor antagonist, and it was also inhibited by BMY7378, a selective \( \alpha_{1D} \)-subtype antagonist. Moreover, treatment with an \( \alpha_{1B} \)-subtype antagonist, chloroethylclonidine, did not

\[ \text{Fig. 4. Effects of increasing doses of WB4101 (A) and BMY7378 (B) on vasoconstrictor responses to 10}^{-8} \text{ mol methoxamine (ME) of isolated rat common carotid arteries. The control vasoconstrictor responses were 14} \pm 1.0 \text{ mmHg (n = 8) for WB4101 and 9} \pm 2.0 \text{ mmHg (n = 9) for BMY7378. PP, perfusion pressure. *P<0.05 and **P<0.01.} \]
influence the noradrenaline- and phenylephrine-induced responses in the used doses, indicating the absence of the \(\alpha_{1D}\)-adrenoceptor subtype. It has been recognized that WB4101 has its blocking properties for not only the \(\alpha_{1A}\)-adrenoceptor but also the \(\alpha_{1D}\)-adrenoceptor subtype (16, 17), and the methoxamine-induced constrictions were readily inhibited by WB4101. Therefore, it is not ruled out that there are functional \(\alpha_{1D}\)-adrenoceptors but no functional \(\alpha_{1A}\) subtype in rat common carotid arteries.

Recently, in the isolated canine splenic artery Yang and Chiba (18) demonstrated that 1) the vasoconstrictor responses to exogenously injected noradrenaline were readily blocked by WB4101 but not by chloroethylclonidine, and 2) periarterial nerve stimulation-induced vasoconstrictions were readily blocked by chloroethylclonidine but not by WB4101. Thus, they suggested that the extrajunctional \(\alpha_{1}\)-adrenoceptor subtype is the \(\alpha_{1A}\) receptor, and the junctional \(\alpha_{1}\)-subtype is the \(\alpha_{1B}\)-receptor in the nerve vascular smooth muscle junctional region. However, as reported before (1), in isolated rat common carotid arteries, tyramine induced only a slight vasostriction. We tried to obtain periarterial nerve stimulation-induced vasoconstrictions in the rat common carotid artery, but failed to obtain stable and obvious constrictions. Thus, it is possible to consider that \(\alpha_{1B}\) subtypes, which may exist the nerve-smooth muscle junctional area, may be scarcely present in rat common carotid artery.

REFERENCES
