CHARACTERIZATION OF NOREPINEPHRINE-INDUCED CONTRACTILE RESPONSE IN ISOLATED RABBIT BASILAR ARTERY

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It is well known that α-adrenoceptors have been classified into the α₁-type in postsynaptic sites and the α₂-type in presynaptic sites (1). Prazosin and yohimbine are selective antagonists of α₁- and α₂-adrenoceptors, respectively (2). Norepinephrine (NE) exerts a non-selective agonistic action at each type of α-adrenoceptors (3, 4). Recent reports present evidence that postsynaptic α₂ as well as α₁-adrenoceptors mediate vasoconstrictor response (5, 6). It has been demonstrated that the dose-response curve for NE shows a biphasic shape in isolated rabbit basilar artery (7-9). However, the mechanism by which the dose-response curve for NE shows the biphasic shape is not yet clear. Therefore, we studied the mechanism by which the dose-response curve for NE shows the biphasic shape, with special reference to the difference in extracellular calcium dependence of contraction between the two phases of the dose-response curve for NE.

A helically cut strip of basilar artery (about 20 mm in length) isolated from male albino rabbits weighing 2.0 to 3.0 kg was suspended in a 20 ml organ bath filled with Krebs buffer solution (NaCl, 118; KCl, 4.5; CaCl₂, 2.5; MgSO₄, 1.0; KH₂PO₄, 1.0; NaHCO₃, 25; glucose, 6.0 mM) being maintained at 32±0.5°C and gassed with a mixture of 95% O₂ and 5% CO₂. Tension development was measured isometrically using a force displacement transducer (San-Ei, Type 45196). Resting tension was adjusted to 0.25 g. After the basilar artery preparation was allowed to equilibrate for at least 1 hr, cumulative dose-response curves for NE were obtained. Antagonists or smooth muscle relaxants were added 5 min before the addition of NE. Exchange of normal buffer solution for lower calcium solutions (Ca²⁺; 0.25 mM, 0.1 mM) was performed 15 min before the addition of NE. Drugs used in this study were papaverine hydrochloride (Tokyo Kasei), D 600 (methoxyverapamil, Knoll), 1-norepinephrine bitartrate (Wako Junyaku), prazosin hydrochloride (Pfizer) and yohimbine hydrochloride (Wako Junyaku). All the drugs were used as solutions in distilled water.

In the isolated rabbit basilar artery, the dose-response curve for NE showed a biphasic shape which corresponded to the phase for lower NE concentrations and that for higher NE concentrations. In this paper, a part of the dose-response curve obtained by NE in the doses ranging from 10⁻⁸ M to 10⁻⁵ M was termed the “lower NE dose component”, and the other obtained by NE in the doses ranging from 10⁻⁶ M to 3×10⁻³ M was called the “higher NE dose component”.

D 600 (3×10⁻⁸ M and 10⁻⁷ M) reduced NE-induced contraction in a dose dependent manner. The reduction was especially marked in the lower NE dose component (Fig. 1A). When the contraction induced by 10⁻⁵ M
NE in the absence of D600 was defined as 100%, the contraction induced by $10^{-5}$ M NE in the presence of D600 at $3 \times 10^{-9}$ M and that at $10^{-7}$ M were 31±7% (mean±S.E. of 5 experiments) and 13±2% (N=5), respectively. On the other hand, when the contraction induced by $3 \times 10^{-3}$ M NE in the absence of D600 was defined as 100%, the contraction by $3 \times 10^{-3}$ M NE in the presence of D600 at $3 \times 10^{-8}$ M and that at $10^{-7}$ M were 57±2% (N=5) and 32±4% (N=5), respectively (Fig. 1A). In the presence of D600 at each concentration, the % contraction induced by $10^{-5}$ M NE was significantly smaller than that induced by $3 \times 10^{-3}$ M NE (P<0.01).

The NE-induced contraction was also reduced by lowering calcium ion concentrations in the buffer solution (low calcium solution, Fig. 1B). the reduction was marked in the lower NE dose component. In this regard, the effect of lowering calcium concentrations was similar to that of adding D600. There was a correlation between the degree of lowering calcium concentrations and the magnitude of reduction of the contraction. When the contraction by $10^{-5}$ M NE in normal buffer solution was defined as 100%, the contraction by $10^{-5}$ M NE in 0.25 mM calcium solution and that in 0.1 mM calcium solution were 27±9% (N=5) and 9±3% (N=5), respectively. On the other hand, when the contraction induced by $3 \times 10^{-3}$ M NE in normal buffer solution was defined as 100%, the contraction induced by $3 \times 10^{-3}$ M NE in 0.25 mM calcium solution and that in 0.1 mM calcium solution were 77±12% (N=5) and 35±5% (N=5), respectively (Fig. 1B). In each concentration of calcium, the % contraction induced by $10^{-5}$ M NE was significantly smaller than that induced by $3 \times 10^{-3}$ M NE (P<0.01).

Papaverine, at $10^{-5}$ M and $3 \times 10^{-5}$ M, reduced NE-induced contraction in a dose dependent manner. According to the same way of calculation described above, the contractions by $10^{-5}$ M NE in the presence of papaverine at $10^{-5}$ M and that at $3 \times 10^{-5}$ M were 46±8% (N=5) and 11±3% (N=5), respectively. The contractions induced by $3 \times 10^{-3}$ M NE in the presence of papaverine at $10^{-5}$ M and that at $3 \times 10^{-5}$ M were 40±8% (N=5) and 17±5% (N=5), respectively (Fig. 1C). Thus, papaverine reduced the contraction of both lower and higher NE dose components in essentially the same way.

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**Fig. 1.** The effect of D600 (A: control, ● $3 \times 10^{-8}$ M, ▲ $10^{-7}$ M), low calcium solution (B: control, ● 0.25 mM Ca$^{2+}$, ▲ 0.1 mM Ca$^{2+}$) and papaverine (C: control, ● $10^{-5}$ M, ▲ $3 \times 10^{-5}$ M) on the dose-response curve of norepinephrine in isolated rabbit basilar arteries. Each value is presented as a mean ±S.E. Ordinates: % of maximum contraction. Abscissas: -log concentration (M) of norepinephrine. Maximal tension in control responses were 447±21 mg in (A), 432±33 mg in (B) and 478±27 mg in (C).
degree, whereas D 600 and low calcium solution reduced the lower NE dose component more effectively than the higher NE dose component.

Only the lower NE dose (10\(^{-5}\) M)-induced contraction was inhibited by prazosin (10\(^{-7}\) M, which is a concentration to show a competitive antagonism to the NE response (2)) by 29±5% (N=4), but the reduction was slight and statistically insignificant (Fig. 2A). In all the ranges of concentrations of NE, the NE contraction was not inhibited by yohimbine (10\(^{-7}\) M) (Fig. 2B). Addition of yohimbine alone caused a weak but sustained contraction that was unaffected by prazosin (10\(^{-7}\) M) (N=5).

In this study, it was confirmed that the dose-response curve for NE in the isolated rabbit basilar artery preparation showed a biphasic shape which consisted of the lower and higher NE dose components. In addition, the contraction of this preparation induced by higher concentrations of NE (10\(^{-5}\) M–3\times10\(^{-3}\) M) was less sensitive to lowering calcium ion concentrations in the buffer solution (low calcium solution) than the contraction induced by lower concentrations of NE (10\(^{-8}\) M–10\(^{-6}\) M). A similar result was obtained when D 600, a calcium entry blocker, was used. On the other hand, when papaverine, a nonspecific smooth muscle relaxant, was used, both lower and higher NE dose components were reduced in essentially the same degree. These results suggest that in this smooth muscle preparation, the dose-response curve for NE consists of two different mechanisms of contraction. It is considered that the lower NE dose component may be dependent on influx of extracellular calcium and the higher NE dose component dependent on release of intracellularly stored calcium because the former was markedly reduced by lowering calcium ion concentration in the buffer solution or calcium entry blocker, D 600, whereas the latter was little affected by lowering calcium ion concentration in the buffer solution or by D 600 (Fig. 1AB).

A part of our results is consistent with the findings of McCalden et al. (10) who reported that NE (10\(^{-5}\) M)-induced contraction of rabbit basilar artery was dependent on extracellular calcium. Prazosin, a selective \(\alpha_1\)-adrenoceptor antagonist (2), slightly reduced the lower NE dose component, whereas yohimbine, a selective \(\alpha_2\)-adrenoceptor antagonist, neither affected the lower nor higher NE dose components. From these results, it is suggested that the higher NE dose component of this tissue is mediated through neither \(\alpha_1\)- nor \(\alpha_2\)-adrenoceptors. Furthermore, because reduction of the lower NE dose component by prazosin was very small and because the dose-response curve was not shifted to the right by prazosin, it is also suggested that the contraction in the lower NE dose component is mediated through neither classical \(\alpha_1\)- nor \(\alpha_2\)-adrenoceptors, but may be mediated by
unknown α-adrenoceptor subtypes or other unknown mechanisms. It is necessary to obtain more evidence to clarify this problem. The mechanism of yohimbine-induced contraction was not investigated in this study.

References