Effects of N\textsuperscript{G}-Nitro-L-Arginine on \(\alpha\)-Agonists-Induced Contraction of Aortae from Wistar Kyoto Rats and Stroke-Prone Spontaneously Hypertensive Rats

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Abstract

Differences in the influences of endothelium-derived nitric oxide (NO) on \(\alpha\)-agonists-induced contraction in the aortae of spontaneously hypertensive and normotensive rats were studied by blocking NO synthesis with N\textsuperscript{G}-nitro-L-arginine (L-NNA). L-NNA potentiated the contraction induced by noradrenaline. The potentiation was smaller in the preparation from stroke-prone spontaneously hypertensive rats (SHRSP) than in the preparation from Wistar Kyoto rats (WKY). Similar potentiation was observed in the contraction induced by phenylephrine; the potentiation was also smaller in the preparation from SHRSP. \(\alpha\)- agonists, clonidine and UK-14304 induced dose-dependent contraction only in the presence of L-NNA. The dose-response curves for \(\alpha\)-agonists in SHRSP aorta were different from those in WKY aorta; the maximum tension was observed at the concentration of 10\textsuperscript{-6} M in the preparation from WKY, while the contraction further increased up to 10\textsuperscript{-4} M in the preparation from WKY. Noradrenaline, clonidine and UK-14304 but not phenylephrine induced relaxation which was blocked by L-NNA. The relaxation was impaired in the preparation from SHRSP in greater extent than that by acetylcholine. It is suggested that basic or noradrenaline-stimulated NO release from endothelium decreased in the preparation from SHRSP and that \(\alpha\)-adrenoceptor of both the endothelium and smooth muscle may be altered in the preparation from SHRSP.

Key words: \(\alpha\)-agonists, contraction, relaxation, endothelium, L-nitroarginine (L-NNA)

Introduction

Noradrenaline-induced contraction of vascular smooth muscle is depressed in the presence of intact endothelium (Cocks and Angus, 1983; Carrier and White, 1985; Murakami et al., 1985; Miller and Vanhouotte, 1985; Cohen et al., 1988; Nyborg, 1990; Osugi et al., 1990; Dohi et al., 1990; Vinet et al., 1991; Vo et al., 1992; Sunano et al., 1992; see also Martin, 1988 and Miller et al., 1988). The depression is thought to be mediated by endothelium derived relaxing factor (EDRF) released spontaneously or by the stimulation with noradrenaline (Martin et al.,
The EDRF which is involved in the depression of noradrenaline-induced contraction is regarded as nitric oxide (NO) derived from L-arginine, since the depression can be blocked by agents which block the NO synthesis (Vo et al., 1992; Kaneko and Sunano, 1993). However, reports on the adrenoceptors involved in the release of EDRF are still controversial (see Miller et al., 1988). We have recently reported that the stimulation of both $\alpha_1$- and $\alpha_2$-adrenoceptors by noradrenaline can induce the release of NO in Wistar Kyoto (WKY) rats (Kaneko and Sunano, 1993).

In aorta of spontaneously hypertensive rats, it has been known that endothelium-dependent relaxation is impaired (Konishi and Su, 1983; Sunano et al., 1989; Clozel et al., 1990; Sunano et al., 1992). Accordingly, the depression of the contraction by endothelium is attenuated in the blood vessels of these hypertensive rats (Osugi et al., 1990). In the present study, effects of the inhibition of NO synthesis by N$^\circ$-nitro-L-arginine (L-NNA) (Moore et al., 1990; Vargas et al., 1991) on noradrenaline-induced contractions of aortae from stroke-prone spontaneously hypertensive rats (SHRSP) and control normotensive Wistar Kyoto rats (WKY) were compared. In addition, the effects of L-NNA on the contraction by $\alpha_1$- and $\alpha_2$-adrenoceptor agonists were also compared separately. SHRSP was used as a hypertensive model, since endothelium-dependent relaxation has been known to be more impaired than that of spontaneously hypertensive rats (SHR) (Sumuno et al., 1989).

Methods

Sixteen weeks old SHRSP and age matched WKY were used in the present experiments. They were originally obtained from Dr. Okamato and successively bred in our animal facility. Systolic blood pressure of these rats were measured by means of tail cuff method. Prior to the measurement, rats were warmed at 40°C for 5 to 10 minutes.

The rats were killed by bleeding from carotid artery under anesthesia with ethyl ether. Aorta was excised from thoracic cavity and ring preparations (1 mm long) were made from descending part. The preparations were immersed in a modified Tyrode’s solution described below. In about one tenth of the preparations, endothelium was removed by rubbing inner surface of the lumen with a small piece of soft rubber band.

The composition of the modified Tyrode’s solution was as follows (mM): NaCl, 137; KCl, 5.4; CaCl$_2$, 2.0; MgCl$_2$, 1.0; NaHCO$_3$, 11.9; NaH$_2$PO$_4$, 0.4; glucose, 5.6. The solution was equilibrated with a gas mixture of 95% O$_2$ and 5% CO$_2$ at 37°C. High-K$^+$ Tyrode’s solution was made by replacing NaCl in the modified Tyrode’s solution with equimolar KCl.

The preparations were mounted in organ baths (10 ml) filled with the modified Tyrode’s solution under stretch tension of 800 mg and tension changes were measured isometrically with force-displacement transducer (Shin-koh, Japan). It was ascertained that further increase in stretch tension caused the decrease in the endothelium-dependent relaxation in the preparations from both SHRSP and WKY, while the amplitude of noradrenaline-induced contraction increased. Prior to the experiments, two successive initiation of high K$^+$ (50 mM)-induced contraction of the duration of 15 min and of the interval of 15 min were performed. These procedures were required to obtain constant results in following experiments. Since the
influence of endothelium on noradrenaline-induced contraction differs time-dependently and the influence was greater in the early phase after the administration of the drug, the dose-response experiments in the present studies were performed within 30 minutes (early phase). The relaxing effects of drugs were observed by adding the drugs cumulatively to the preparation precontracted in the presence of $10^{-9}$ M ONO-11113. The effect of phenylephrine was observed in the presence of $10^{-6}$ M propranolol, and the effects of noradrenaline, clonidine and UK-14304 were observed in the presence of $10^{-6}$ M prazosin and $10^{-6}$ M propranolol.

Following drugs were used in the present experiments: noradrenaline bitartrate (Sigma, St. Louis, MO, U.S.A.), phenylephrine hydrochloride (Sigma, St. Louis, MO, U.S.A.), clonidine (Sigma, St. Louis, MO, U.S.A.), UK-14304 (5-bromo-6-[2-imidazoline-2-yl amino]-quinoxaline, courtesy of Pfizer limited, Sandwich, Kent, U.K.), ONO-11113 (STa2, 9.11-epithio-11, 12-methano-TXA2, courtesy of Ono pharmaceutical Co., Osaka, Japan), N⁶-nitro-L-arginine (L-NNA, Sigma, St. Louis, MO, U.S.A.) prazosin hydrochloride (Sigma St. Louis, CO, U.S.A.), yohimbine hydrochloride (Sigma, St. Louis, MO, U.S.A.) and propranolol hydrochloride (Sigma, St. Louis, MO, U.S.A.).

Obtained values were expressed as mean±s.e.m. and differences between values in the presence and absence of L-NNA and between values of WKY and SHRSP aortae were analyzed separately by Student’s t-test considering $P<0.05$ as significant.

Results

Body weight and blood pressure of SHRSP and WKY

Body weight of 16 weeks old SHRSP and age matched WKY were 245±3.0 g (n=38) and 323±3.7 g (n=52), respectively. The body weight of SHRSP of our animal facility was slightly smaller than that of WKY ($P<0.001$). Blood pressure of SHRSP was 245±1.1 mmHg (n=38) and that of WKY was 135±1.0 mmHg (n=52), being significantly higher in the former ($P<0.001$).

Difference in the effects of L-NNA on noradrenaline-induced contraction between aortae from SHRSP and WKY

Noradrenaline-induced contraction of aorta was depressed in the presence of endothelium. The depression was less in the preparation from SHRSP when compared with that from WKY. Similar effects as those of removal of endothelium were observed by the treatment with $10^{-4}$ M L-NNA (Fig. 1). It was ascertained that the concentration of L-NNA was sufficient to block the endothelium-dependent relaxation induced by acetylcholine in the preparations both from SHRSP and WKY. L-NNA of this concentration caused the elevation of basal tone, lowered threshold concentration of noradrenaline for contraction and increased maximal tension development by noradrenaline in both preparations. The difference between the contraction in the absence and presence of L-NNA was smaller in the preparation from SHRSP than that from WKY.
**Phenylephrine-, clonidine- and UK-14304-induced contractions**

L-NNA potentiated the contraction by phenylephrine in the same manner as the removal of endothelium. The potentiation was similar to that observed with noradrenaline; elevation of the basal tension, lowered threshold concentration and increased maximal tension development. The difference between the contractions in the absence and presence of L-NNA was also smaller in the preparation from SHRSP (Fig. 2).

Clonidine induced no contractile response in the endothelium-intact preparations both from SHRSP and WKY (Fig. 3). In endothelium-removed preparation, clonidine induced contraction. Similar contraction was observed in the preparation treated with $10^{-4}$ M L-NNA (Fig. 3). The contraction amplitude increased dose-dependently from $10^{-7}$ to $10^{-4}$ M in the preparation from WKY. In the preparation from SHRSP, the contraction was smaller, achieved its maximum at the concentration on $10^{-6}$ M and no further increase in the contraction amplitude
Fig. 3. Contraction induced by clonidine in the presence of L-NNA in aortae of WKY and SHRSP. mean±s.e.m. of 6 to 10 preparations. Others are the same as those in Fig. 1. Note the difference in the dose–response curve in the presence of L-NNA.

Fig. 4. The effects of L-NNA on the UK-14304-induced contraction of aortae from SHRSP and WKY. mean±s.e.m. of 4 to 9 preparations. Others are the same as those in Fig. 1 and Fig. 3.

was observed even when the concentration of the drug was increased (Fig. 3). Similar results were obtained with UK-14304, an \( \alpha_2 \)-adrenoceptor stimulant, except for the slight increase in the contraction amplitude at higher concentration of the drug both in the preparations from WKY and SHRSP (Fig. 4).

*Relaxation by \( \alpha \)-agonists*

Relaxations by \( \alpha \)-agonists and acetylcholine were observed with the preparations precontracted in the presence of 10\(^{-9}\) M ONO-11113, a thromboxane analogue (Table 1). Noradrenaline induced a dose–dependent relaxation in the presence of prazosin and propranolol; the maximum relaxation was observed at 10\(^{-4}\) M and the amplitude of the relaxation was significantly less in the preparation from SHRSP when compared with that in the preparation from WKY.

Phenylephrine in the concentrations up to 10\(^{-4}\) M did not cause the relaxation but caused
Table 1. Maximum relaxation of aorta from WKY and SHRSP by various \(\alpha\)-agonists and acetylcholine.

| drugs  | concen. | relaxation |
|--------|--------|------------|
| NA     | \(10^{-4}\) | 26.2±5.9 | 8.1±2.4* |
| Phenyleph | \(10^{-5}\) | -3.4±0.8 | -19.5±4.0* |
| UK     | \(10^{-4}\) | 37.4±4.3 | 8.5±2.6* |
| Cloni  | \(10^{-4}\) | 23.2±3.8 | 12.5±2.6* |
| Ach    | \(10^{-4}\) | 74.2±3.3 | 47.6±2.1* |

Relaxations were expressed as percentages of the precontraction induced by \(10^{-9}\)M ONO-11113. Mean±s.e.m. of 8 to 23 preparations from 5 to 10 rats. NA, Phenyleph, UK, Cloni, Ach indicate noradrenaline in the presence of prazosin and propranolol, phenylephrine in the presence of propranolol, UK-14304 and clonidine in the presence of prazosin and propranolol, and acetylcholine, respectively. Concentration (M) of above drugs at which the maximum relaxation was observed. WKY and SHRSP indicate the preparation from WKY and SHRSP, respectively. * indicates significant difference between the value of WKY (\(P<0.001\)).

Discussion

Noradrenaline-induced contraction of aortae both from SHRSP and WKY was depressed in the presence of endothelium. Spontaneous release of EDRF, release stimulated by noradrenaline, or both of these release are thought to be the cause of the depression as has been reported (Cooks and Angus, 1983; Carrier and White, 1985; Miller and Vanhoutte, 1985; Martin et al., 1986; Cohen et al., 1988; Nyborg, 1990; McGrath et al., 1990; Vinet et al., 1991; see Martin, 1988 and Miller et al., 1988).

In aorta of spontaneously hypertensive rats, reports on endothelium-dependent relaxation are in agreement that the relaxation was impaired when compared with those of normotensive rats (Konishi and Su, 1983; Sunano et al., 1989; Clozel et al., 1990; Shimamura et al., 1991; Sunano et al., 1992), although the discussions on the mechanisms of the impairment are still controversial. As the cause of the impairment, decreased EDRF release, disturbance of diffusion of EDRF from endothelium to smooth muscle, decreased sensitivity to EDRF and/or corelease of endothelium-derived contracting factor (EDCF) are proposed (see Luscher and Vanhoutte, 1988). We have previously reported that the impairment of endothelium-dependent relaxation in aorta was stronger as the blood pressure of the rats was higher (Sunano et al., 1989). The impairment was, therefore, greater in the preparation from SHRSP when compared with that from SHR. It was also demonstrated that the endothelium was structurally slight contraction of the preparations both from WKY and SHRSP in the presence of propranolol. \(\alpha\)-agonists, clonidine and UK-14304, caused the relaxation of preparations in the presence of prazosin and propranolol; the relaxation was impaired in the preparation from SHRSP. Acetylcholine induced largest relaxing response which was also impaired in the preparation from SHRSP. The relaxations by all these drugs were blocked by \(10^{-4}\)M L-NNA.
damaged in SHRSP aorta (Sunano et al., 1993) indicating that the release of EDRF was decreased in SHRSP aorta due to structural damage. Then, the decreased depressing action of endothelium on the agonists-induced contraction in the preparation from SHRSP would be brought about by the decreased release of EDRF. In the present experiment, the involvement of EDRF in contractions induced by $\alpha$-agonists was studied by the treatment with L-NNA.

EDRF has been known to be NO which is synthesized from L-arginine (Furchgott and Vanhoutte, 1989; Ignarro, 1989; Moncada et al., 1991) and the synthesis of NO can be blocked by some agents including L-NNA used in the present experiment (Moore et al., 1990; Vargas et al., 1991). It was demonstrated in the present experiment with aorta of WKY and SHRSP that L-NNA inhibited endothelium-dependent depression of noradrenaline-induced contraction. Similar effects of blocking NO synthesis has been reported with $N^\omega$-monomethyl-L-arginine (L-NMMA) in mesenteric resistance artery of WKY and SHR (Dohi et al., 1990). We have observed that this concentration of L-NNA blocked completely the relaxation induced by acetylcholine in the aorta both from WKY and SHRSP (Kaneko and Sunano, 1993). It can thus be concluded that the depression of noradrenaline-induced contractions was mediated by the NO released from endothelium. The decreased influence of L-NNA on noradrenaline-induced contraction in the preparation from SHRSP may then be thought to be due to the decrease in NO release as postulated in mesenteric resistance artery of SHR (Dohi et al., 1990).

When decrease in the release of NO by noradrenaline is discussed, changes in the receptors in SHRSP should also be considered. It was reported that the stimulation of both of $\alpha_1$- and $\alpha_2$-adrenoceptors of endothelium can release EDRF, although the reports are still controversial (Carrier and White, 1985; Cohen et al., 1985; Murakami et al., 1985; Miller and Vanhoutte, 1985; Nyborg, 1990; McGrath et al., 1990; Vinet et al., 1991; see Miller et al., 1988). We have observed that noradrenaline can stimulate both $\alpha_1$- and $\alpha_2$-adrenoceptors of endothelium and induces the release of NO (Kaneko and Sunano, 1993). Then, changes in these adrenoceptors can also be a cause of the alteration of the release of NO. In the present experiments, it was shown that L-NNA potentiated phenylephrine-induced contraction markedly in the preparation both from SHRSP and WKY. The result indicates that $\alpha_1$-adrenoceptor is involved in the release of NO in both preparations. Involvement of $\alpha_1$-adrenoceptor of endothelium in the depression of agonist-induced contraction has also been reported in the other vascular tissues (Carrier and White, 1985; McGrath et al., 1990; Vinet et al., 1991). Thus, the decreased influence of endothelium on noradrenaline-induced contraction may be explained by the change in $\alpha_1$-receptor in endothelium.

However, more marked influence of L-NNA was observed in the contraction by clonidine or UK-14304, $\alpha_2$-receptor agonists, in the preparation both from SHRSP and WKY. As have previously been reported (Kaneko and Sunano, 1993), clonidine induced no contraction unless L-NNA was applied. Similar effects were observed by the removal of endothelium (Carrier and White, 1985; Vinet et al., 1990) or by the application of methylene blue (Vinet et al., 1990). It has also been reported that the stimulation of $\alpha_2$-adrenoceptor was more effective to release EDRF than the stimulation of $\alpha_1$-adrenoceptor (Cocks and Angus, 1983; Miller and Vanhoutte, 1985). It can therefore be assumed that the amount of NO released by the stimulation with $\alpha_2$-adrenoceptor agonist is sufficient to depress totally the contraction of smooth muscle induced
by the $\alpha_2$-adrenoceptor agonist of the same concentration.

In the present experiments, it was shown that noradrenaline can induce the relaxation in the preparations both from WKY and SHRSP; the relaxation was impaired in the latter. It was also demonstrated that clonidine or UK-14304 induced relaxation and that the relaxation was impaired in the preparation from SHRSP. The ratio of the impairment of the relaxation (46% and 78% respectively is the relaxation by clonidine and UK-14304) was greater than that induced by acetylcholine (36%). Thus, the involvement of the difference in $\alpha_2$-adrenoceptor in the impairment of the relaxation by noradrenaline and in the reduced modifying effect of endothelium in noradrenaline-induced contraction is suggested. However it was not possible to compare the involvement of endothelial $\alpha_2$-adrenoceptor in the depression of the contraction by $\alpha_2$-agonists between the preparation from SHRSP and WKY, because endothelium blocked totally the contraction induced by the same adrenoceptor of the smooth muscle in both preparations. The amplitude of contraction induced by clonidine or UK-14304 in the presence of L-NNA was smaller and apparent ED$_{50}$ was also smaller in the preparation from SHRSP. This may indicate the difference in $\alpha_2$-adrenoceptor of vascular smooth muscle in hypertensive and normotensive rats (Medgett et al., 1984).

Although phenylephrine failed to induced relaxation, this may be due to the stronger contractile action of the drug on smooth muscles, since it was demonstrated in the present experiment that the contraction induced by phenylephrine was also depressed by NO.

In conclusion, endothelium dependent depression of noradrenaline-induced contraction was impaired in aorta of SHRSP when compared with that of WKY as observed by the application of L-NNA. Similar depression was observed in the contractile response to phenylephrine, clonidine and UK-14304. Noradrenaline, clonidine and UK-14304 induced relaxation. These relaxations were impaired in the preparation from SHRSP when compared to that from WKY, being more prominent than the relaxation by acetylcholine. It is suggested that the impairment of the endothelium-dependent depression was brought about by the decreased release of NO, and that changes in the adrenoceptors, especially $\alpha_2$-adrenoceptor of endothelium may be involved in this change.

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