Difference in Effects of Stretch on Depressive Effect of Endothelium-Derived Nitric Oxide on Noradrenaline- and High-K⁺-Induced Contractions between the Aortae from Normotensive and Spontaneously Hypertensive Rats

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Abstract

Difference in effects of stretch tension on endothelium-derived nitric oxide (EDNO)-dependent depression of noradrenaline (NA)- and high-K⁺-induced contraction between the aortae from normotensive Wistar Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) was studied. NA-induced contraction in preparations both from WKY and SHRSP was augmented in the presence of Nω-nitro-L-arginine (L-NNA). This augmentation was minimized when the spontaneous tone, which was more prominent in preparations from SHRSP, was subtracted and the effects of L-NNA became less prominent in preparations from SHRSP. The effects of L-NNA were maximal at the stretch tension of 15 mN and, then, decreased as stretch tension increased in both preparations when the spontaneous tone was subtracted. The effects of L-NNA were less prominent when the contraction was initiated by high-K⁺, although the effects of stretch on high-K⁺-induced contraction were similar to that of NA-induced contraction. These results suggested 1) that both NA- and high-K⁺-induced contractions are depressed by EDNO, 2) that the release of EDNO induced by high-K⁺ is less than that by NA, 3) that increase in stretch tension decreases the release of EDNO, and 4) that the depressive effect of EDNO on contraction is impaired in the aorta of SHRSP.

Key words: aorta, endothelium-derived nitric oxide, stretch tension, stroke-prone spontaneously hypertensive rats

Introduction

Endothelium releases various factors spontaneously and in response to various stimulations

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These factors influence the contraction to various stimulations. Among these endothelium-derived factors, nitric oxide (NO) has been known as a endothelium-derived relaxing factor (EDRF). It has also been known that endothelium-derived NO (EDNO) depresses the spontaneous contraction (Sunano et al., 1996; Sunano et al., 1997) or contraction to various agonists (see Vanhoutte et al., 1986; Miller et al., 1988). Spontaneous (basal) release of EDNO or the release in response to stimulation, which was used to initiate contraction, is responsible for the depression of the contraction. NO is synthesized in endothelium from L-arginine by NO synthase (Palmer et al., 1988), and this synthesis can be blocked by drugs such as Nω-monomethyl-L-arginine (Rees et al., 1989), Nω-nitro-L-arginine methyl ester (Rees et al., 1990) or Nω-nitro-L-arginine (L-NNA, Moore et al., 1990).

It has been reported that both endothelium-dependent relaxation and depression of contraction were impaired in blood vessels of spontaneously hypertensive rats (Sunano et al., 1989; Matsuda et al., 1995; see Lüscher, 1988; Lüscher and Vanhoutte, 1990; Vanhoutte and Boulanger, 1995). The contraction and endothelium-dependent relaxation of blood vessels were affected by stretch tension of the preparations (Sekiguchi et al., 1996; Dainty et al., 1990; Katusic et al., 1987; Rubanyi et al., 1990). If the release of EDNO is altered by the change in stretch tension, then the influence of EDNO on the contraction would vary at various stretch tension. It is also possible that the influence of endothelium on the contraction at various stretch tension is different between the preparations from normotensive and spontaneously hypertensive rats.

In the present experiments, the effects of stretch tension on the endothelium-dependent depression of contraction of the aorta to noradrenaline (NA) and elevated K+ were investigated. In addition, the differences in the effects of stretch tension between the preparations from normotensive Wistar Kyoto rats (WKY) and stroke-prone spontaneously hypertensive rats (SHRSP) were also studied.

**Methods**

WKY and SHRSP were used in the present experiments. These rats were purchased from Shimizu Laboratory Supplies Co. Ltd. (Kyoto, Japan) at the age of 5 weeks and fed in our animal facility until they were sacrificed to the experiment (16 weeks of age) under the conditions of 22°C, 50% humidity and 12 h light-and-dark cycle. Normal chow (SP, Funabashi, Japan) and tap water were given freely. The care in treating these animals was taken according to the guiding principles for the care and use of laboratory animals approved by The Japanese Pharmacological Society.

Blood pressure of the rats was measured by means of tail-cuff method. Prior to the measurement, rats were warmed at 40°C for 10 min. The procedure was required to obtain exact value of blood pressure in this method.

Contractions of preparations and influence of endothelium on the contraction were observed using the aortae of the rats described above. The rats were killed by bleeding from vena cava under anesthesia with CO2 gas, and the aortae were dissected from thoracic cavity. Adhesive fat and connective tissues of the thoracic aorta were carefully removed in a modified Tyrode’s
solution of the following composition, and ring preparations of 1 mm in width were made.

The modified Tyrode's solution had following composition (mM): NaCl, 137; KCl, 5.4; CaCl₂, 2.0; MgCl₂, 1.0; NaHCO₃, 11.9; NaH₂PO₄, 0.4; glucose, 5.6; and Ca-ethylenediamine tetraacetic acid (Ca-EDTA), 0.02; equilibrated with a gas mixture of 95% O₂ + 5% CO₂ at 37°C. K⁺-Tyrode's solution was made by replacing all NaCl to equimolar KCl, and high-K⁺ solutions containing different concentrations of K⁺ were made by mixing the modified Tyrode's solution with the K⁺-Tyrode's solution at appropriate rate. Ca-free solution was made by omitting Ca²⁺ from the modified Tyrode's solution.

Two tungsten wires of 30 µm in diameter were inserted into the lumen of the ring preparation and the preparation was mounted on an organ bath with one tungsten wire. The other tungsten wire was connected to a mechano-electronic transducer (Minebea, Nagano, Japan) so that isometric tension changes could be measured. The stretch tension of the preparation was adjusted to various levels from 8 to 50 mN in the Ca-free solution where the spontaneous tone of the smooth muscle disappeared.

After the equilibration in the modified Tyrode's solution for 60 min, the preparation was subjected to high-K⁺-induced contraction by changing the solution to high-K⁺ Tyrode's solution containing 50 mM K⁺ for 20 min. This procedure was repeated two times with a interval of 20 min. The procedures were required to obtain the constant value of the contraction in following experiment and the second contraction was used to normalize the following tension changes. As a final procedure of the experiments, 10⁻⁵ M verapamil and 10⁻⁴ M papaverine were added to induce the complete relaxation and the all tensions were measured from this level. The influence of EDNO was studied by observing the contractions 30 min after the application of 10⁻⁴ M L-NNA.

Drugs used in the present experiments were: noradrenaline bitartrate (NA, Wako, Osaka, Japan), Nω-nitro-L-arginine (L-NNA, Sigma, St. Louis, USA), verapamil hydrochloride (Wako), papaverine hydrochloride (Wako) and Ca-ethylenediamine tetraacetic acid (Ca-EDTA, Dojindo, Kumamoto, Japan).

Obtained data were expressed as means ± SE. The n values indicate the number of animals or preparations. These data were analyzed by Student’s t test. Concentration-response curves were analyzed by two-way ANOVA followed by Bonferroni/Dunn’s test for post hoc, then, each points were analyzed by Student’s t test in the case that there was the significant difference between the two curves. P values less than 0.05 were considered to be significant difference.

Results

Body weight and systolic blood pressure of rats

Body weight and systolic blood pressure of WKY and SHRSP at the age of 16 weeks were shown in Table 1. The body weight of SHRSP was significantly smaller than that of WKY. The systolic blood pressure of SHRSP was significantly and markedly higher than that of WKY.

Spontaneous tension development at various stretch tension

Endothelium-intact preparations from SHRSP showed spontaneous tension development
(spontaneous tone), which increased as the stretch tension increased, while the spontaneous tone and the increase by stretch were not obvious in preparations from WKY (Fig. 1). The spontaneous tone was augmented in the presence of L-NNA (10^{-4} M). The augmentation was also greater in preparations from SHRSP (Fig. 1).

**Effect of L-NNA on NA-induced contraction at various stretch tension**

At the stretch tension of 8 mN, NA-induced contraction was greater in preparations from WKY than that in those from SHRSP (Fig. 2). The contraction was markedly augmented in the presence of L-NNA in both preparations. The augmentation became greater as the concentrations of NA increased. The effects of L-NNA were greater in preparations from WKY.

When the stretch tension increased to 30 mN, NA-induced contraction of preparations both from WKY and SHRSP were augmented (Fig. 3). However, the augmentation of contraction by L-NNA was reduced in preparations from WKY (Figs. 3a and 3b). In preparations from SHRSP, the augmentation by L-NNA was more prominent as compared with that at the stretch tension of 8 mN (Fig. 3c). However, the augmentation became less prominent when spontaneous tone was subtracted (Fig. 3d).

Fig. 4 shows the maximal contraction in the absence and presence of L-NNA at various stretch tension. In preparations from WKY, the augmentation of contraction by L-NNA was

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Table 1  Body weight and systolic blood pressure of rats at the age of 16 weeks.

|            | Body weight (g) | Systolic blood pressure (mmHg) |
|------------|-----------------|-------------------------------|
| WKY (n=30) | 394.7 ± 3.5     | 140.9 ± 1.8                   |
| SHRSP (n=41)| 271.7 ± 5.5**   | 238.6 ± 3.6**                  |

Mean ± SE of the values obtained from rats of number indicated in parentheses. Asterisks indicate significant differences from respective value obtained with WKY (**, P<0.001).
Effect of stretch and EDNO on contraction

maximal at the stretch tension of 15 mN, and decreased as the stretch tension increased (Figs. 4a and 4b). In preparations from SHRSP, the augmentation was maximal at the stretch tension of 50 mN when spontaneous tone was included (Fig. 4c). However, the effect of L-NNA decreased by subtracting spontaneous tone, and was suppressed by the increase in stretch tension (Fig. 4d). When spontaneous tone was subtracted, the effect of L-NNA was maximal at the stretch tension of 15 mN in both preparations, but markedly smaller in preparations from SHRSP.

Since the developed tension was smaller in preparations from SHRSP, the difference in the effect of L-NNA between both preparations was corrected by normalizing the maximal tension developed in the presence of L-NNA as 100% (Fig. 5). The augmentation of NA-induced contraction, especially that at low stretch tension, was still smaller in preparations from SHRSP as observed when the tone of preparations was subtracted (Figs. 5b and 5d).

Fig. 2. Concentration-response curves for noradrenaline (NA)-induced contraction at the stretch tension of 8 mN in the absence and presence of L-NNA (10^-4 M). (a), (b): the curves taken with preparations from WKY. (c), (d): the curves taken with preparations from SHRSP. (a), (c): the tension developed by NA containing spontaneous tone. (b), (d): the tension developed by NA subtracting spontaneous tone. Values were expressed as mean ± SE of 9 to 12 preparations. Asterisks indicate significant differences from respective value obtained in the absence of L-NNA (*, P<0.05; **, P<0.001).
Effect of L-NNA on high-K⁺-induced contraction at various stretch tension

At the stretch tension of 8 mN, contraction induced by the elevation of K⁺ concentration was greater in preparations from WKY than that in those from SHRSP (Fig. 6). In the presence of L-NNA, K⁺-induced contraction increased in both preparations. The effects of L-NNA were greater in preparations from WKY. The difference of the effects of L-NNA between preparations from WKY and SHRSP was more prominent when the spontaneous tone was subtracted (Figs. 6b and 6d). In the case, no significant difference between in the absence and presence of L-NNA was observed in preparations from SHRSP (Fig. 6d).

Fig. 7 shows the high-K⁺-induced contraction at the stretch tension of 30 mN. Increasing the stretch tension augmented both the contraction and the effects of L-NNA. However, when spontaneous tone was subtracted, the effects of L-NNA became similar to those at the stretch tension of 8 mN in both preparations (Figs. 7b and 7d).

The relationship between stretch tension and L-NNA-induced augmentation of the maximal...
Effect of stretch and EDNO on contraction amplitude of high-K⁺-induced contraction, observed at 70 mM K⁺, was summarized in Figs. 8 and 9. The tendency of this relationship was similar to that in NA-induced contraction, however, the effects of L-NNA were less prominent in both preparations than those in NA-induced contraction.

Discussion

Blood vessels relax in response to various agents such as acetylcholine, bradykinin or calcium ionophore. The relaxation of blood vessels is brought about by a factor or factors released from endothelium (Furchgott and Zawadzki, 1980). In addition to these relaxing agents, the agents, which induce contraction of vascular smooth muscle, also induce the release of these relaxing factors (see Lüscher and Vanhoutte, 1990; Furchgott and Vanhoutte, 1989). Moreover, it has been known that vascular endothelium also releases these factors.
spontaneously (Martin et al., 1986; Dainty et al., 1990; see Vanhoutte et al., 1986). Then, it can be assumed that the release of EDRF would depress the contraction of smooth muscle by various stimulations as has been postulated (Martin et al., 1986; Kaneko and Sunano, 1993; see Miller et al., 1988).

It has been reported that the release of these factors is affected by the stretch of the preparation (Dainty et al., 1990). We have also reported that contraction and endothelium-dependent relaxation of rat aorta are affected by stretch, and that the effects of stretch were different between preparations from WKY and SHRSP (Sekiguchi et al., 1996). The difference would not be caused by the difference in the sensitivity to NA used to initiate the precontraction, since it has been reported that no difference could be observed in the effect of stretch on sensitivity to NA between preparations from WKY and SHR (Coskinas and Price, 1987; and present study).

It was shown in the present experiment that not only agonist-induced contraction but also the spontaneous contraction (tone) was affected by stretch. The prominent effect of stretch observed in preparations from SHRSP, especially in the presence of L-NNA, may be explained
Effect of stretch and EDNO on contraction

by different effect of stretch on the membrane potential between vascular smooth muscles from WKY and SHRSP as reported in cerebral arteries (Harder et al., 1985), and this should be taken into consideration, when the effects of stretch on agonist-induced contraction are studied.

The augmentation of NA-induced contraction of the aortae from WKY and SHRSP by L-NNA, shown in the present experiments, is thought to be brought about by the inhibition of NO release from endothelium. In these preparations, it has been shown that endothelium-dependent relaxation to acetylcholine can be blocked completely by L-NNA (Sekiguchi et al., 1996), indicating that the relaxation is caused entirely by NO from endothelium (Moore et al., 1990; Ishii et al., 1990; Mayer et al., 1993). Smaller depression of NA- and high-K+-induced contractions by EDNO in preparations from SHRSP compared to those from WKY may be explained by reduced release of NO, as we have reported previously (Matsuda et al., 1995; Matsuda et al., 1998). In support of this, acetylcholine-induced relaxation of aortic preparations has been shown to be impaired in preparations from SHRSP (Sekiguchi et al., 1996). An alternative explanation for the smaller depression of contraction in preparations from SHRSP is an involvement of coreleased endothelium-derived contracting factor (EDCF). Such an
involvement of EDCF has been reported to be a cause of the impairment of endothelium-dependent relaxation of the aorta of hypertensive rats (Kato et al., 1990; Ito et al., 1991; Lüscher and Vanhoutte, 1986). In the present experiment, however, the involvement of NO was mainly studied, since the effect was almost completely abolished by L-NNA and the effect of L-NNA was almost same as those observed by the removal of endothelium.

It was shown in the present experiment that the contraction of the aorta induced by NA was depressed by EDNO as reported previously (Kaneko and Sunano, 1993; Matsuda et al., 1995; Matsuda et al., 1998). EDNO would be released by the stimulation with NA (Eglême et al., 1984; Carrier and White, 1985) or spontaneously (Martin et al., 1986; Murakami et al., 1985). We have reported that stimulation both α1- and α2-receptors of the endothelium with NA can cause the release of EDNO (Kaneko and Sunano, 1993). Similar results have been reported by Eglême et al. (1984) and Carrier and White (1985).

In the present experiment, it was shown that the augmentation of NA-induced contraction by
L-NNA decreased as stretch tension increased, and that the effect of stretch was marked in preparations from WKY when the spontaneous tone was subtracted. These results are concomitant with our previous paper on the effect of stretch on endothelium-dependent relaxation (Sekiguchi et al., 1996). In the previous paper, we reported that the endothelium-dependent relaxation decreased as stretch tension increased, and that the effect of the stretch was more prominent in preparations from WKY compared with those from SHRSP. In preparations from SHRSP, the stretch tension-dependent decrease in the effect of L-NNA was not observed and the effect of L-NNA rather increased when the spontaneous tone was included. This may be due to the increase in the stretch-induced spontaneous tone in the preparations reported previously (Sekiguchi et al., 1996).

Dainty et al. (1990) have reported that increase in precontraction by stretch is a cause of the decreased endothelium-dependent relaxation. It was shown in the present experiment that the NA-induced contraction increased as the stretch tension increased. This may also be a cause of the decreased effect of L-NNA by the stretch, although it remains uncertain whether this leads
to the decreased release of EDNO. The increased corelease of EDCF by the stretch can also be a cause of the reduced modification of contraction by EDNO (Sekiguchi et al., 1996).

It is uncertain whether or not the change in membrane potential of endothelial cells induces the release of EDRF (see Pearson and Vanhoutte, 1993). However, the result, that high-K⁺-induced contraction was augmented by L-NNA, indicates that NO is also released in response to the application of high concentrations of K⁺; the membrane of endothelium is thought to be depolarized under this condition (Johns et al., 1987). It has been reported that high-K⁺ does not increase but rather decrease Ca²⁺ influx in cultured endothelial cells (Johns et al., 1987). Moreover, intracellular Ca²⁺ concentration was not increased but rather decreased by the application of high-K⁺ (Laskey et al., 1990). Nevertheless, L-NNA augmented high-K⁺-induced contraction, indicating the involvement of NO in high-K⁺-induced contraction. The basal release of NO and its inhibition by L-NNA may be an explanation for this. However, the effect of L-NNA increased as K⁺ concentration increased, indicating that NO was released in response to K⁺. Thus, the mechanisms of high-K⁺-induced release of NO remain to be further investigated. In any case, the involvement of NO would be less in the contraction by high-K⁺ than that by
receptor stimulation, since the effect of L-NNA was weaker in the contraction by high-K⁺ as compared with that in the contraction induced by NA.

The release of NO through this process is also thought to be less affected by the stretch. Again, the larger stretch-dependent augmentation of high-K⁺-induced contraction in the preparations from SHRSP can mainly be explained by stretch-dependent increase in the spontaneous tone (present results and Sekiguchi et al., 1996), since it was minimized when the tone was subtracted.

The possibility, that EDNO is exhausted to depress the tone in preparations from SHRSP so that the depression of NA- or high-K⁺-induced contraction is reduced, is unlikely, since acetylcholine can still induce relaxation of large amplitude in NA-precontracted preparations from SHRSP (Sekiguchi et al., 1996).

In conclusion, both NA- and high-K⁺-induced contractions of rat aortae were augmented by L-NNA; the augmentation being greater in the former than in the latter. The stimulation of a receptor on the endothelium by NA or depolarization of endothelial cell membrane by high-K⁺ induce the release of NO and attenuate the contraction to these stimulation. The effect of stretch on the augmentation by L-NNA of contraction was greater in NA-induced contraction compared with high-K⁺-induced contraction. When compared between preparations from WKY and SHRSP, the effects of L-NNA were greater in preparations from WKY. The effects of stretch can be explained both by the effect on NO release and that on the spontaneous tone.

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