Blood Pressure and Age-Dependent Changes of Endothelium-Dependent Tension Oscillations in Different Strains of Spontaneously Hypertensive Rats

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Abstract

The influences of blood pressure and age of spontaneously hypertensive rats on endothelium-dependent tension oscillation of aortic preparation were studied. Rats with different blood pressures, normotensive Wistar Kyoto rats (WKY), spontaneously hypertensive rats (SHR), stroke-prone SHR (SHRSP) and malignant type of SHRSP (M-SHRSP), were used. The effects of antihypertensive treatment of SHRSP on the tension oscillation were also studied. High doses of noradrenaline induced tension oscillations in endothelium-intact preparations of all strains of young rats. The rate of the occurrence of the tension oscillation decreased age-dependently. The decrease was faster when the blood pressure of the rats was higher. Application of acetylcholine in the presence of noradrenaline induced a relaxation and tension oscillations, both of which were negatively dependent on age and blood pressure. Antihypertensive treatment of hypertensive rats with hydralazine or captopril prevented a decrease in incidence of the tension oscillation. These influences of age and blood pressure as well as antihypertensive treatments on the tension oscillation resembled those on the endothelium-dependent relaxation and are thought to be brought about by functional changes of the endothelium.

Key words: tension oscillation, hypertensive rats, age, endothelium, antihypertensive treatment

Introduction

Aortae of rats exhibit tension oscillation in response to the stimulation by noradrenaline at high concentrations (Sunano et al., 1992a). The tension oscillation is endothelium-dependent and thought to be brought about by certain factor(s) released from endothelium as has been proposed in hamster aorta (Jackson, 1988) and canine basilar artery (Katusic et al., 1988). The endothelium-dependent tension oscillations are rarely observed in the aortae from spontaneously hypertensive rats with established hypertension, although the smooth muscle exhibits...
endothelium-independent tension oscillations (Sunano et al., 1992a) as has been reported in the smooth muscle of the tail artery (Lamb et al., 1985; Meyers et al., 1985; Bruner et al., 1986). In addition, the endothelium-dependent tension oscillations were also observed during acetylcholine-induced relaxation. Thus, it is suggested that the functional change of endothelium plays a role in the change in endothelium-dependent tension oscillations.

It has been known that endothelium-dependent relaxation is impaired in the blood vessels of spontaneously hypertensive rats (see Luscher and Vanhoutte, 1988). The impairment is dependent on age and degree of hypertension (Sunano et al., 1989). The results may indicate that functions of endothelium of the aorta from spontaneously hypertensive rats are impaired depending on the age and degree of hypertension. It has also been reported that the impairment of the endothelium-dependent relaxation of hypertensive rats could be prevented by the antihypertensive treatment of these rats (Luscher et al., 1987; Van de Vooorde et al., 1988; Sunano et al., 1989, Clozel et al., 1990; Sunano et al., 1992b, Sunano et al., 1993). In the present studies, influences of age and blood pressure on the endothelium-dependent tension oscillation and the effects of chronic antihypertensive treatment of hypertensive rats were examined using strains from the same origin with different blood pressure.

**Materials and methods**

Wistar Kyoto rats (WKY), spontaneously hypertensive rats (SHR), stroke-prone SHR (SHRSP) and malignant SHRSP (M-SHRSP) were used in the present study. It should be mentioned that all strains of hypertensive rats used in the present experiments were derived originally from WKY (Okamoto and Aoki, 1963; Okamoto et al., 1974; Okamoto et al., 1986). These rats were bred and fed in our institute under conditions of constant temperature (23°C) and light-dark cycle of 12 hs. Blood pressure of the rats was measured every two weeks by the tail cuff method. Animals were sacrificed at 8, 12, 16 and 20 weeks of age. These rats were killed by cutting carotid artery under anaesthesia with diethylether.

Aorta of the animals was dissected from thoracic cavity and ring preparations of 1 mm width were made from the aorta using fine scissors for microsurgery. Great care was taken not to damage endothelium during these procedures. The preparations were incubated in a modified Tyrode's solution of following composition (mM): NaCl, 137; KCl, 5.4; CaCl₂, 2.0; MgCl₂, 1.0; NaHCO₃, 1.19; NaH₂PO₄, 0.4; glucose, 5.6; Ca(II)-EDTA, 0.026, equilibrated with 95% O₂ and 5% CO₂ at 37°C. High-K Tyrode's solution containing 50 mM K⁺ was made by replacing NaCl in the modified Tyrode's solution with equimolar KCl.

Two thin tungsten wires (30 μm in diameter) were inserted in the lumen of the preparation, and one tungsten wire was connected to an organ bath and the other, to a force-displacement transducer (Shin-koh, Nagano, Japan), so that the tension changes were observed isometrically. The basal stretch tension of 800 mg was chosen, since the maximum endothelium-dependent relaxation was observed at this stretch tension (Sekiguchi et al., 1996). The preparations were equilibrated in the modified Tyrode's solution at least 1 h prior to the application of stimulants. Then, they were subjected to two successive high-K-induced contractures changing the incubation medium from the modified Tyrode's to high-K-Tyrode's solution containing 50 mM K⁺.
The procedures were required to obtain constant results in the subsequent experiments.

The endothelium-dependent tension oscillations were observed in the presence of $10^{-5}$ M noradrenaline, since the tension oscillations were observed at high concentration of the drug (Sunano et al., 1992a). Endothelium-dependent relaxation and associated tension oscillations were observed by adding $10^{-5}$ M acetylcholine to the precontracted preparations in the presence of $5 \times 10^{-7}$ M noradrenaline. The amount of the added stock solution containing these drugs were less than 1 percent of the incubation medium.

Antihypertensive treatment of animals were performed giving hydralazine or captopril to SHRSP from the age of weaning (5 week old) to the age of 16 weeks. Hydralazine was administrated by mixing the drug in drinking water and captopril, by mixing in the chow. In another series, the treatment was started at the age of 16 weeks and continued to the age of 26 weeks. The dose of the drugs was controlled to maintain blood pressure level close to that of WKY. It ranged from 5.9 mg/kg/day to 8.6 mg/kg/day and from 22 mg/kg/day to 55 mg/kg/day, respectively in hydralazine− and captopril−treatment. The treated animals were sacrificed at the age of 16 and 26 weeks, respectively.

Drugs used in the present experiments were; noradrenaline bitartrate (Sigma, St. Louis, MO, USA), verapamil hydrochloride (Sigma), calcium disodium ethylenediaminetetraacetate (Ca(II)−EDTA, Wako, Osaka, Japan), hydralazine hydrochloride (Sigma) and captopril (courtesy of Bristol−Meyers Squibb).

Obtained values were analyzed by ANOVA and P values smaller than 0.05 were considered to be significantly different.

**Results**

**Blood pressure of animals**

Age−dependent changes of blood pressure of different strains of rats used in the present experiments were shown in Fig.1. The blood pressure elevated age−dependently; the elevation being prominent in following order: WKY<SHR<SHRSP<M−SHRSP. The blood pressure of M−SHRSP after 12 weeks of age could not be measured because of high rate of stroke incidence.

![Fig.1. Age−dependent elevation of systolic blood pressure in WKY and spontaneously hypertensive rats of different strains (SHR, SHRSP, M−SHRSP). Difference in blood pressure among different strains are statistically significant (p<0.001). Blood pressure of M−SHRSP after 12 weeks of age could not be measured because of high rate of stroke. Mean of 10 to 20 rats. S.E.s are smaller than diameter of each symbol.](attachment:figure1.jpg)
Endothelium-dependent tension oscillation and age of rats

In the present experiments, noradrenaline of the concentration of $10^{-5}$ M was applied, since it has been shown that endothelium-dependent tension oscillations can be initiated by high concentration of drug (Sunano et al., 1992a). Noradrenaline of this concentration induced contraction and tension oscillations. The tension oscillations were abolished by the removal of the endothelium.

The rate of the occurrence of the tension oscillations decreased age-dependently even in the preparation from WKY. The rate of the decrease was steeper when the blood pressure of the rats was higher (Table 1). In the preparations from SHRSP of ages older than 16 weeks, for example, only a few preparations exhibited the tension oscillation (Fig. 2, Table 1). In the preparation from M-SHRSP of the age of 12 weeks, it was shown that the rate of the occurrence of the tension oscillations was already decreased markedly when compared with that of the preparation from WKY. The rate of the occurrence of older M-SHRSP could not

Table 1. Rates of preparation which showed tension oscillations in response to $10^{-5}$ M noradrenaline

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>26</th>
<th>16E(−)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>10/11 (91%)</td>
<td>17/20 (85%)</td>
<td>61/74 (82%)</td>
<td>13/24 (54%)</td>
<td>33/57 (58%)</td>
<td>0/45 (0%)</td>
</tr>
<tr>
<td>SHR</td>
<td>8/8 (100%)</td>
<td>10/13 (78%)</td>
<td>16/33 (49%)</td>
<td>10/25 (40%)</td>
<td>7/24 (29%)</td>
<td></td>
</tr>
<tr>
<td>SHRSP</td>
<td>16/18 (89%)</td>
<td>9/13 (69%)</td>
<td>6/46 (13%)</td>
<td>4/21 (19%)</td>
<td>0/29 (0%)</td>
<td>0/46 (0%)</td>
</tr>
<tr>
<td>M-SHRSP</td>
<td>22/22 (100%)</td>
<td>8/18 (44%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers of preparations which showed tension oscillations/Number of preparations examined. Preparations were made from 5 to 38 rats. Percentages of the preparation which showed tension oscillations were also indicated in the parenthesis. E(−) indicates the endothelium-denuded preparation. $^{a}(P<0.05)$, $^{b}(P<0.01)$, $^{c}(P<0.001)$: significant difference from age-matched WKY, respectively. $^{d}(P<0.05)$, $^{e}(P<0.01)$, $^{f}(P<0.001)$: significant difference from 8 week-old rats of each strain, respectively. $^{g}(P<0.05)$, $^{h}(P<0.001)$: significant difference from the endothelium-intact preparations from rats of the same age, respectively.

Fig. 2. Contraction and tension oscillation induced by noradrenaline in aorta from WKY and SHRSP at ages of 12 and 20 weeks. Noradrenaline (NA) of $10^{-5}$ M was applied. Note the change in the contraction curve in aorta from 20 week old SHRSP.
Fig. 3. Influence of age on relaxing response to acetylcholine and tension oscillation in SHRSP aorta. Contractions and relaxations were induced by $5 \times 10^{-7}$ M noradrenaline (NA) and $10^{-5}$ M acetylcholine (Ach), respectively. Numbers on the left of traces represent the ages of SHRSP (week). Typical tracings of the response of preparations from 8 week-old SHRSP which showed twitch-like contraction (top trace) and slow fluctuation of tension (second trace). Note age-dependent decrease of the relaxation and the occurrence of the tension oscillation.

Table 2. Rates of preparations which showed tension oscillations in response to acetylcholine in the presence of noradrenaline

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>8</th>
<th>12</th>
<th>16</th>
<th>20</th>
<th>26</th>
<th>16E(—)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strain</td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>WKY</td>
<td>11/11 (100%)</td>
<td>21/22 (96%)</td>
<td>36/41 (88%)</td>
<td>36/44 (82%)</td>
<td>49/56 (88%)</td>
<td>0/88 (0%)</td>
</tr>
<tr>
<td>SHR</td>
<td>10/10 (100%)</td>
<td>9/10 (90%)</td>
<td>28/32 (88%)</td>
<td>24/33 (71%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHRSP</td>
<td>12/14 (86%)</td>
<td>11/14 (79%)</td>
<td>5/42* (12%)</td>
<td>3/24* (13%)</td>
<td>2/24* (8%)</td>
<td>0/79* (0%)</td>
</tr>
<tr>
<td>M-SHRSP</td>
<td>16/25 (64%)</td>
<td>7/17 (41%)</td>
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</table>

$10^{-5}$ M acetylcholine was applied in the presence of $5 \times 10^{-7}$ M noradrenaline, so that the maximum endothelium-dependent relaxation could be observed. *($P < 0.05$), **($P < 0.001$): significant difference from age-matched WKY, respectively. *($P < 0.001$): significant difference from 8 week-old rats of each strain. *($P < 0.05$), **($P < 0.001$): significant difference from the endothelium-intact preparation from rats of the same age, respectively. Others are same as those in Table 1.

be examined because of high rate of the stroke incidence.

_Tension oscillation induced by acetylcholine_

The application of acetylcholine ($10^{-5}$ M) to the preparation contracted in the presence of $5 \times 10^{-7}$ M noradrenaline induced a relaxation of the preparation. The relaxation was impaired in the preparations from spontaneously hypertensive rats as reported previously (Sunano et al., 1989). During the relaxation, tension oscillations of high amplitude were often observed (Fig. 3). Both the rate of the occurrence and the amplitude of the tension oscillations were higher than those induced by noradrenaline alone. The rate of the occurrence decreased also depending on the age and the degree of hypertension (Table 2).

_Effects of antihypertensive therapy_

The treatment of SHRSP with hydralazine or captopril from the age of weaning (5 weeks old, early treatment) attenuated the development of hypertension. The blood pressure of hydralazine- and captopril-treated SHRSP at age of 16 weeks was $131 \pm 2.2$ mmHg ($n=6$) and
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Table 3. Effects of antihypertensive treatment on tension oscillations induced by noradrenaline in SHRSP

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>16</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>6/46</td>
<td>0/29</td>
</tr>
<tr>
<td></td>
<td>(13%)</td>
<td>(0%)</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>52/63*</td>
<td>18/50**</td>
</tr>
<tr>
<td></td>
<td>(83%)</td>
<td>(36%)</td>
</tr>
<tr>
<td>Captopril</td>
<td>26/29*</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(90%)</td>
<td></td>
</tr>
</tbody>
</table>

*SHRSP were treated from 6 weeks to 16 weeks of age. **SHRSP were treated from 16 weeks to 26 weeks of age. At least 7 rats were used in each treatment. *P<0.001: significant difference from age-matched untreated SHRSP. **P<0.001: significant difference from 16 week-old Hydralazine-treated SHRSP. Others are same as those in Table 1.

Table 3. Effects of antihypertensive treatment on tension oscillations induced by noradrenaline in SHRSP

<table>
<thead>
<tr>
<th>Age (weeks)</th>
<th>16</th>
<th>26</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>5/45</td>
<td>3/24</td>
</tr>
<tr>
<td></td>
<td>(11%)</td>
<td>(13%)</td>
</tr>
<tr>
<td>Hydralazine</td>
<td>34/40*</td>
<td>21/27**</td>
</tr>
<tr>
<td></td>
<td>(85%)</td>
<td>(78%)</td>
</tr>
<tr>
<td>Captopril</td>
<td>21/46**</td>
<td></td>
</tr>
<tr>
<td></td>
<td>(46%)</td>
<td></td>
</tr>
</tbody>
</table>

*SHRSP were treated from 6 weeks of age. **SHRSP were treated from 16 weeks of age. At least 8 hydralazine-treated SHRSP were used in each experiment. *P<0.001: significant difference from age-matched untreated SHRSP. **P<0.001: significant difference from 16 week-old hydralazine-treated SHRSP. Others are same as those in Table 1.

155±1.6 mmHg (n=6), respectively. Although the latter value is slightly higher than that of WKY (136±0.8 mmHg, n=20), it is significantly lower than that of untreated SHRSP (245±1.2 mmHg, n=20, p<0.001). Effects of antihypertensive treatment after the hypertension has fixed (16 weeks old, late treatment) was also studied by treating SHRSP with hydralazine. The treatment lowered the elevated blood pressure. The blood pressure of the treated SHRSP at the age of 26 weeks (after 10 weeks treatment) was 154±5.0 mmHg (n=7), being significantly lower than that of the untreated SHRSP of the same age (265±1.0 mmHg, n=7, p<0.001).

The rate of the occurrence of noradrenaline-induced tension oscillations of the preparations from SHRSP was increased by these treatment of the rats and similar to that of the preparations from WKY (Table 3). The rate of the occurrence of the tension oscillations of the preparation which underwent the late treatment also increased but the rate was still lower than that of the preparations from WKY of the same age (Table 3). Age-dependent decrease of acetylcholine-induced tension oscillations of the preparations from SHRSP was also prevented by antihypertensive treatment from weaning period (Table 4). The rate of the occurrence of the acetylcholine-induced tension oscillations was restored also by the late treatment of SHRSP, although the rate was still lower than that of WKY (Table 4).
Blood Pressure and Tension Oscillation

Discussion

The tension oscillations of aortae of rats can be divided into two types: endothelium-dependent type and myogenic type (Sunano et al., 1992a; Jackson, 1988; Katusic et al., 1988; Meyers et al., 1985; Lamb et al., 1985). The tension oscillations observed both in the presence of high concentration of noradrenaline and during the relaxation by acetylcholine of noradrenaline-precontracted preparation were abolished by the removal of endothelium. It can therefore be considered that the tension oscillations observed in the present experiments were endothelium-dependent one. Most probably, the release of factor(s) from endothelium is the cause of the occurrence of the tension oscillations.

Since the tension oscillations are abolished by the removal of extracellular Ca or by the addition of Ca-antagonist (Sunano et al., 1992a), Ca-influx through voltage-dependent channel would initiate them. Then, it is suggested that oscillatory changes in the membrane potential occur in response to the application of noradrenaline as in the other vascular smooth muscle (Lamb and Webb, 1988a; 1988b). In addition, Harder (1987) has reported that endothelium plays a role in the initiation of repetitive action potentials of cerebral arterial smooth muscle. It is thus possible that endothelium releases the factor(s) which induces the oscillatory membrane potential changes and this leads to the tension oscillations.

Although such factors have not been identified, all of the relaxing, contracting and hyperpolarizing factors can be a candidate of the factor which causes oscillatory changes of membrane potential changes, since it has been reported that they can affect the membrane potential (Tare et al., 1990; Harder et al., 1989; Chen and Suzuki, 1990). In addition, unknown(s) factor which induces the oscillatory changes of membrane potential may be involved. Thus, it was indicated that the factor(s) may cause the oscillatory membrane potential changes which lead to the oscillation of voltage-dependent Ca current, although the identificant of the factor(s) remained to be further investigated.

In aorta of spontaneously hypertensive rats, endothelium-dependent relaxation has been known to be impaired (see Luscher and Vanhoutte, 1988), indicating that the functional abnormalities of endothelium. We have reported that the impairment of endothelium-dependent relaxation became more prominent as the blood pressure of the rat elevated (Sunano et al., 1989). In the present experiment, it was shown that the rate of the occurrence of the tension oscillations was decreased in the preparation from hypertensive rats and the decrease was more prominent in the preparations from the rats with higher blood pressure. Thus, coincidence of the impairments of endothelium-dependent relaxation and the decrease in the occurrence of the tension oscillations was indicated.

Coincidence was also shown in the age-dependent decrease of the occurrence of the tension oscillations. The rate of the occurrence of the tension oscillations decreased as the age of the rats increased, similarly to the endothelium-dependent relaxation (Sunano et al., 1989; Moritoki et al., 1986; 1988). The results thus indicate that the decrease of the endothelium-dependent tension oscillations is brought about by blood pressure- and age-dependent change of the function of endothelium. The decrease in the release of the factor(s) or the interaction of the factors is thought to be the cause of the decrease in the occurrence of the tension oscillations.
oscillations as in the impairment of endothelium-dependent relaxation.

The endothelium-dependent tension oscillations were observed during the relaxation by acetylcholine. Since the tension oscillations as well as the relaxation were not observed in the preparation from which endothelium was removed, it can be considered that the tension oscillations induced by acetylcholine were also induced by the release of factor(s) from endothelium. In the aorta of hypertensive rats, the occurrence of the tension oscillations was reduced depending on the degree of hypertension. Again, we have observed that the endothelium-dependent relaxation by acetylcholine was impaired in the aorta of hypertensive rats depending on the degree of hypertension (Sunano et al., 1989). In addition, the age-dependency of the decrease in the rate of the occurrence of the acetylcholine-induced tension oscillations also resembled to the endothelium-dependent relaxation (Sunano et al., 1989). Thus, the relationship between the endothelium-dependent relaxation and the tension oscillation was strongly suggested.

Similarity of the endothelium-dependent tension oscillation to the endothelium-dependent relaxation was also demonstrated in the preparation from SHRSP which had received antihypertensive treatment. It has been shown that the treatment brought about the prevention of the impairment of the endothelium-dependent relaxation (Sunano et al., 1993; Sunano et al., 1992; Luscher et al., 1987; Clozer et al., 1990). The increased rate of the occurrence of the tension oscillations in aorta from treated SHRSP may be due to the prevention of the impairment of the endothelial function. The result of the late treatment indicates that the normal endothelial function can be restored by chronic antihypertensive treatment even when the treatment was started after the hypertension was fixed. Then, the decreased rate of occurrence of endothelium-dependent tension oscillations would be the secondary changes of endothelium due to maintained hypertension. We have obtained similar results in the experiments of antihypertensive treatment on endothelium-dependent relaxation (Sunano et al., 1993). Thus, the coincidence between the endothelium-dependent tension oscillation and relaxation was shown also in the antihypertensive treatment experiments.

In conclusion, aorta of the rats exhibits endothelium-dependent tension oscillations in response to high concentration of noradrenaline or by acetylcholine. The rate of the occurrence of the tension oscillations was reduced in the preparation from spontaneously hypertensive rats. The decrease was dependent on the blood pressure and age of the rats. Chronic antihypertensive treatment of hypertensive rats prevented the decrease and restored the tension oscillations. These results resembled the endothelium-dependent relaxation and indicate that the release of the endothelium-derived factor(s) which is (are) involved in the initiation of the tension oscillations, is altered in the aorta of hypertensive rats.

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