Laboratory Studies

Central Attenuation of Aortic Baroreceptor Reflex in Prehypertensive DOCA-Salt-Loaded Rats

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SUMMARY To determine whether the arterial baroreceptor reflex can act to oppose the development of hypertension, deoxycorticosterone acetate (DOCA)-salt hypertension was produced in sinoaortic-denervated and sham-operated rats. Systolic blood pressure measured by tail cuff started to increase in both sinoaortic-denervated and sham-operated rats 7 days after DOCA treatment, and the hypertension developed identically in both denervated and sham-operated rats. These findings suggest that the baroreceptor reflex cannot act against the development of hypertension. To determine whether the baroreceptor reflex is attenuated before the development of hypertension, bradycardic and sympathoinhibitory responses to i.v. injections of norepinephrine were examined. Bradycardic and sympathoinhibitory responses were significantly smaller in DOCA-salt-treated rats in both prehypertensive (5th day after DOCA-salt treatment) and hypertensive stages (21st day after treatment). In urethane-anesthetized DOCA-loaded and control rats on the 5th day after treatment, aortic depressor nerve stimulation elicited frequency-dependent depressor and bradycardic responses accompanied by inhibition of sympathetic nerve activity in both DOCA-loaded and control rats. However, those responses were significantly smaller in DOCA-loaded rats than in control rats. These results suggest that the central component of the baroreceptor reflex mediated by the aortic depressor nerve is impaired before hypertension develops and that this impairment may contribute to the development of hypertension in DOCA-salt-treated rats. (Hypertension 12: 259-266, 1988)

KEY WORDS • arterial baroreceptor reflexes • deoxycorticosterone acetate-salt hypertension • sinoaortic denervation • aortic depressor nerve • sympathetic nerve activity

ARTERIAL baroreceptor reflex function is impaired in established human hypertension as well as in animals with experimentally induced hypertension.1-4 This impairment has been understood as "resetting" following the development of hypertension. However, recent studies have shown that baroreceptor reflex sensitivity is reduced before hypertension develops in hypertension-prone Dahl salt-sensitive rats.5-11 Baroreceptor reflex control of heart rate has also been found to be impaired in borderline hypertensive subjects12, 13 and normotensive young subjects with a family history of essential hypertension.14 A decrease in arterial distensibility and adaptation of the baroreceptors themselves15-17 have been demonstrated as underlying mechanisms of this abnormality. These changes are not likely to be responsible for baroreceptor reflex changes before the development of hypertension, since arterial distensibility and baroreceptor changes can occur after the development of hypertension. Recently, the baroreceptor reflex was shown to be attenuated centrally in spontaneously hypertensive rats.18, 19 These reports support the possibility that the attenuation of baroreceptor reflex function could precede and contribute to the development of hypertension. Thus, in this study, we attempted to determine whether the baroreceptor reflex was attenuated centrally in the prehypertensive stage of deoxycorticosterone acetate (DOCA)-salt hypertension, and we also examined the role of baroreceptor reflexes in the development of hypertension.

Materials and Methods

Fifty-four male Wistar rats (Shimizu, Kyoto, Japan), weighing about 150 g, were used for these
experiments. Rats were anesthetized with pentobarbital sodium (35 mg/kg i.p.), and their left kidneys were removed. Thereafter, sinoaortic denervation was performed according to the method of Krieger. After 1 week, sinoaortic-denervated (SAD) and sham-operated rats were given DOCA, 40 mg/kg s.c., and fed high salt diets containing 8.0% NaCl. Control rats (SAD and sham-operated rats) were given saline injections subcutaneously and fed normal diets (0.4% NaCl). In this report the term sham refers to rats that underwent a sham operation for SAD and control refers to rats that were not given the DOCA-salt load. For aortic depressor nerve (ADN) stimulation experiments, six rats received the same treatment with DOCA-salt given to SAD and sham-operated rats. Systolic blood pressure was measured using the tail-cuff method (Narco Biosystems, Houston, TX, USA). To test salt-loading effects, seven rats were fed a high salt diet and six rats were fed a normal salt diet for 5 days. After the last measurements by tail cuff were made, rats were anesthetized with urethane (1.2 g/kg i.p.) and used for ADN stimulation experiments in the prehypertensive stage. Catheters were inserted into a femoral artery for blood pressure recording and into a femoral vein for drug injection. Blood pressure was recorded continuously by connecting the aortic catheter through Tygon tubing to a small-volume-displacement transducer (MVP-290, NESC-Sanei Sokki, Tokyo, Japan) and a heart rate tachograph (NESC-Sanei Sokki), triggered by the phasic blood pressure pulses.

Recording and Analysis of Sympathetic Nerve Activity

The abdominal sympathetic plexus was exposed, and a bipolar stainless steel electrode ( uninsulated tips 1 mm apart) was placed on the major splanchnic nerve between the cardiac and celiac ganglion. The nerves and electrode were immersed in mineral oil to reduce tissue drying. Spike potentials were amplified (P-15 AC amplifier, Grass, Quincy, MA, USA, and biophysoamplifier, NEC-Sanei Sokki) and monitored on a storage oscilloscope (Kikusui Electronics, Tokyo, Japan). To reduce noise during these recordings, spontaneous respiration was abolished by paralyzing skeletal muscles with decamethonium bromide (0.2 mg/100 g i.v.) and connecting the rats to a respirator. Analog signals for aortic pressure, heart rate, and splanchnic nerve activity were recorded continuously on magnetic tape (R-210B, TEAC, Tokyo, Japan).

To quantify nerve activity, original analog signals were played back from tape into an ink-writing recorder and simultaneously fed into an amplitude analyzer to convert individual spikes into uniform pulses and delete background noise. After the nerves were crushed, the low-level control of the window discriminator (spike counter, DIA, Osaka, Japan) was routinely set to filter background noise. The number of individual pulses was counted with a rate analyzer whose output was recorded separately as a histogram and then printed out.

Electrical Stimulation of the Aortic Depressor Nerve

The carotid sinus nerves and ADNs were identified under an operation microscope (Nikon SM 1, Tokyo, Japan). Bilateral carotid sinus nerves, glossopharyngeal nerves, and right aortic depressor nerves were severed. A bipolar stainless steel electrode was placed on the central cut end of the left ADN and immersed in a pool of mineral oil. Rectangular pulses were delivered to the left ADN by a stimulator (Nihon Kohden, Tokyo, Japan) connected to the electrodes by an isolation unit (Model SS201J, Nihon Kohden). The frequency was varied between 3.9 and 31.2 Hz, while the voltage and duration of stimulation were held constant (1.65 V and 3 msec, respectively). The stimulation was continued for 30 seconds.

Statistical Analysis

Data were expressed as the means ± SEM. Significance of differences between means was ascertained using unpaired Wilcoxon's nonparametric method and Student’s t test. Data from more than three groups of rats were analyzed using an analysis of variance, and for F ratios significant at 5% or less, differences between pairs of means were examined using Duncan’s multiple range test. Changes in blood pressure, heart rate, and sympathetic nerve activity when compared with controls were expressed as percent change. Differences were considered significant at the 5% level (p < 0.05).

Results

Time Course of Systolic Blood Pressure and Heart Rate in Conscious Rats

Before DOCA-salt loading was introduced, systolic blood pressure obtained with the tail-cuff method was already significantly higher in SAD rats than in sham-operated rats, and this relation in rats without DOCA-salt treatment persisted throughout the 4 weeks (Figure 1).

When DOCA-salt was given to sham-operated rats, blood pressure started to rise significantly after 7 days of DOCA treatment. In SAD rats, blood pressure rose gradually after DOCA treatment. The development of hypertension was similar in DOCA-treated SAD rats and DOCA-treated sham-operated rats (see Figure 1).

Heart rate was higher in SAD groups with and without DOCA treatment at 1 week, but it did not differ between those groups after 3 weeks (Table 1). In control groups, systolic blood pressure of SAD rats was significantly higher than that of sham-operated rats. These results show that the 5th day after DOCA-salt treatment could be determined as the prehypertensive stage.
Cardiovascular and Sympathetic Inhibitory Responses to Aortic Depressor Nerve Stimulation in Prehypertensive Rats

Systolic blood pressure measured in conscious rats 5 days after DOCA-salt treatment was similar in DOCA-salt–treated and control rats (114 ± 4 vs 110 ± 1 mm Hg, NS). After urethane anesthesia, basal mean blood pressure and heart rate were also similar in both the DOCA-treated and control rats (91 ± 4 vs 86 ± 6 mm Hg, NS; 307 ± 38 vs 418 ± 72 beats/min, NS).

Electrical stimulation of the left ADN elicited frequency-dependent depressor and bradycardic responses accompanied by inhibition of sympathetic nerve activity in both DOCA-salt–treated and control rats (Figure 2). Vasodepressor and bradycardic responses to the ADN stimulation were significantly smaller in DOCA-salt–treated rats than in control rats (Table 2, Figures 3 and 4). Inhibition of sympathetic nerve activity during ADN stimulation was also less in DOCA-salt–treated rats than in control rats (Figure 5; see Table 2).

### Table 1. Heart Rate Changes in Conscious Rats

<table>
<thead>
<tr>
<th>Week</th>
<th>Control-sham (n = 6)</th>
<th>Control-SAD (n = 7)</th>
<th>DOCA-sham (n = 8)</th>
<th>DOCA-SAD (n = 6)</th>
<th>F ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>406 ± 20</td>
<td>432 ± 14</td>
<td>412 ± 8</td>
<td>405 ± 18†</td>
<td>0.8</td>
</tr>
<tr>
<td>1</td>
<td>413 ± 17</td>
<td>456 ± 14*</td>
<td>430 ± 7</td>
<td>493 ± 15</td>
<td>6.4</td>
</tr>
<tr>
<td>2</td>
<td>418 ± 20</td>
<td>442 ± 6</td>
<td>393 ± 10</td>
<td>446 ± 15</td>
<td>3.6</td>
</tr>
<tr>
<td>3</td>
<td>445 ± 20</td>
<td>438 ± 12</td>
<td>408 ± 13</td>
<td>399 ± 17</td>
<td>1.8</td>
</tr>
<tr>
<td>4</td>
<td>406 ± 22</td>
<td>437 ± 14</td>
<td>390 ± 25</td>
<td>439 ± 25</td>
<td>1.3</td>
</tr>
</tbody>
</table>

Values indicate means ± SEM. SAD = sinoaortic denervation; sham = sham operation.

*p < 0.05, †p < 0.01, compared with values in control-sham rats.

†p < 0.01, compared with values in DOCA-sham rats (by Duncan’s multiple range test).
The hypotensive effects elicited by different frequencies of ADN stimulations were significantly correlated, but the regression curve line was significantly blunted \((p < 0.05)\) in DOCA-salt–treated rats (Figure 6). Similar to the changes in blood pressure, both the R-R interval, measured by peak of blood pressure curve, and the decrease in sympathetic nerve discharge slope lines were significantly \((p < 0.05)\) blunted in DOCA-salt–treated rats (Figures 7 and 8).

**Cardiovascular and Sympathetic Nerve Activity Responses to Injected Norepinephrine**

To confirm that sinoaortic denervation was performed successfully, norepinephrine bitartate (100 and 200 ng/100 g) was injected intravenously in the prehypertensive (after 5 days of DOCA-salt treatment) and the hypertensive stage (after 21 days of DOCA-salt treatment). Pressor responses to injected norepinephrine were higher in sham-operated DOCA-salt–treated rats than in control rats in the hypertensive stage (Table 3), but in the prehypertensive stage, these pressor responses were similar between DOCA-salt–treated and control rats (see Table 3). During pressor responses to intravenous injection of NE, decreases in heart rate were remarkable in sham-operated rats, but not in SAD rats in either the hypertensive stage or the prehypertensive stage (Table 4). The bradycardic responses were significantly smaller in sham-operated DOCA-salt–treated rats than in sham-operated control rats, not only in the hypertensive stage (see Table 4) but also in the prehypertensive stage (see Table 4). During pressor responses to intravenous injections of norepinephrine, sympathetic nerve activity was markedly inhibited in sham-operated rats compared with SAD rats. This inhibition of sympathetic nerve activity was significantly smaller in DOCA-salt–treated rats than in controls in both prehypertensive and hypertensive stages (Table 5). In SAD rats, sympathetic nerve activity was not inhibited by norepinephrine-induced pressor responses in either the prehypertensive or the hypertensive stage (see Table 5). Therefore, the sinoaortic denervation

**TABLE 2. Cardiovascular and Sympathetic Nerve Responses Caused by Depressor Nerve Stimulation in Urethane-Anesthetized Prehypertensive DOCA-Salt–Treated Rats and Control Rats**

<table>
<thead>
<tr>
<th>Variable</th>
<th>3.9</th>
<th>7.8</th>
<th>15.6</th>
<th>31.2</th>
</tr>
</thead>
<tbody>
<tr>
<td>BP decrease (mm Hg)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ((n = 6))</td>
<td>8 ± 2</td>
<td>13 ± 3</td>
<td>20 ± 3</td>
<td>36 ± 6</td>
</tr>
<tr>
<td>DOCA-salt ((n = 6))</td>
<td>4 ± 1</td>
<td>7 ± 1</td>
<td>10 ± 1*</td>
<td>19 ± 2*</td>
</tr>
<tr>
<td>HR decrease (beats/min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ((n = 6))</td>
<td>47 ± 20</td>
<td>72 ± 23</td>
<td>131 ± 49</td>
<td>213 ± 69</td>
</tr>
<tr>
<td>DOCA-salt ((n = 6))</td>
<td>13 ± 5</td>
<td>16 ± 4</td>
<td>26 ± 4</td>
<td>45 ± 10</td>
</tr>
<tr>
<td>SNA decrease (spikes/sec)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control ((n = 6))</td>
<td>13 ± 5</td>
<td>18 ± 4</td>
<td>27 ± 4</td>
<td>42 ± 5</td>
</tr>
<tr>
<td>DOCA-salt ((n = 6))</td>
<td>11 ± 2</td>
<td>12 ± 3</td>
<td>16 ± 3*</td>
<td>25 ± 6</td>
</tr>
</tbody>
</table>

Values indicate means ± SEM. BP = blood pressure; HR = heart rate; SNA = sympathetic nerve activity. *\(p < 0.05\), compared with values in control rats.
method was effective. In addition, it showed that baroreceptor reflexes of sympathetic nerve activity were preserved in sham-operated rats. Like the bradycardic responses, the inhibitory reflex of sympathetic nerve activity was also attenuated in the prehypertensive stage and hypertensive stage of DOCA-salt hypertensive rats.

Cardiovascular and Sympathetic Nerve Responses to Aortic Depressor Nerve Stimulation in Salt-Treated Rats

To test the central effect of salt on the baroreceptor reflex, responses to ADN stimulations were examined in rats fed a high salt diet. After 5 days, systolic blood pressures were similar in rats fed a high salt or a normal diet (135 ± 5 vs 128 ± 5 mm Hg; \( n = 7 \)). After anesthetization with urethane, mean blood pressure, heart rate, and basal sympathetic nerve activity were also similar (103 ± 8 vs 106 ± 8 mm Hg; 306 ± 20 vs 290 ± 16 beats/min; 73 ± 6 vs 78 ± 5 spikes/3 sec). Depressor, bradycardic, and sympathoinhibitory responses were not altered by salt treatment (Table 6).

Discussion

Our results show two findings. First, the arterial baroreceptor reflex system does not play any inhibitory role in the development of DOCA-salt hypertension. Second, the baroreceptor reflex was attenuated even before the development of hypertension, and the underlying mechanism of this attenuation was partly dependent on central baroreceptor reflex change.

Sinoaortic denervation produced an elevation of blood pressure in awake rats. After DOCA-salt treatment, blood pressure did not change until the 7th day. Then, blood pressure started to rise during 3 weeks of observation. During the development of DOCA-salt hypertension, the difference in blood pressure between SAD and sham-operated rats disappeared, while in rats without DOCA-salt treatment blood pressure in SAD rats was higher than that in sham-operated rats. These findings indicate that the baroreceptor reflex did not have any inhibitory effect on the development of hypertension in DOCA-salt–treated rats. In other words, the baroreceptor reflex appears to be attenuated during the development of hypertension.

An important role of baroreceptor reflexes in DOCA-salt hypertension has been shown by the fact that aortic baroreceptor deafferentation can render salt-resistant Sabra strain (SBN) rats sensitive to DOCA-salt–induced hypertension.22 Weinstock et al.23 suggested that this rat strain had increased baroreceptor reflex sensitivity that conferred resistance to DOCA-salt hypertension. These findings suggest that attenuation of the baroreceptor reflex may be necessary for the development of DOCA-salt hypertension. The present results indicate clearly that baroreceptor reflexes were attenuated before the development of DOCA-salt hypertension, not only in heart rate control, but also in sympathetic nerve regulation. Impaired baroreceptor reflexes in the prehypertensive stage were also demonstrated in Dahl rats.9-11 However, in spontaneously hypertensive rats, baroreceptor reflex function inhibits the development of hypertension, since debuffered spontaneously hypertensive rats showed an exaggerated development of hypertension.18, 19 Therefore, the contribution of the baroreceptor...
reflex on the development of hypertension may not be a common feature in all models of experimental hypertension.

After the development of hypertension, reduced baroreceptor reflexes are partly due to vessel wall changes. That pressor responses to i.v. injections of norepinephrine were augmented in DOCA-salt hypertensive rats in the hypertensive stage suggests this possibility. However, in the prehypertensive stage, pressor responses to i.v. injection of norepinephrine were similar in DOCA-salt-treated and control rats. Therefore, it is unlikely that vessel wall changes in embedded baroreceptors contribute to reduced baroreceptor reflexes. Bradycardic as well as sympathoinhibitory responses to pressor responses to i.v. injection of norepinephrine were smaller in DOCA-salt-treated rats than in control rats. These findings suggest that the baroreceptor reflex alteration shown in bradycardic responses in normotensive humans with hypertensive parents may be accompanied by alterations of sympathetic nerve control.

Recently, central alteration of baroreceptor reflexes was indicated in spontaneously hypertensive rats and renal hypertension. In the present study, electrical stimulation of the left ADN, which contains few or no fibers from chemoreceptors, elicited frequency-dependent depressor and bradycardic responses accompanied by sympathetic nerve inhibitory responses. In the prehypertensive stage of DOCA-salt hypertension (5th day

<table>
<thead>
<tr>
<th>Stage</th>
<th>BP response (mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td></td>
</tr>
<tr>
<td>Control-sham</td>
<td>16 ± 2</td>
</tr>
<tr>
<td>Control-SAD</td>
<td>27 ± 3*</td>
</tr>
<tr>
<td>DOCA-sham</td>
<td>29 ± 2†</td>
</tr>
<tr>
<td>DOCA-SAD</td>
<td>34 ± 3</td>
</tr>
<tr>
<td>Prehypertensive</td>
<td></td>
</tr>
<tr>
<td>Control-sham</td>
<td>11 ± 3</td>
</tr>
<tr>
<td>Control-SAD</td>
<td>14 ± 3</td>
</tr>
<tr>
<td>DOCA-sham</td>
<td>19 ± 3</td>
</tr>
<tr>
<td>DOCA-SAD</td>
<td>16 ± 5</td>
</tr>
</tbody>
</table>

Values indicate mean changes ± SEM from basal blood pressure. BP = blood pressure; NE = norepinephrine; SAD = sinoaortic denervation; sham = sham operation.

*p < 0.05, †p < 0.01, compared with values in control-sham rats.
Table 4. Heart Rate Response to Intravenous Injection of Norepinephrine in Urethane-Anesthetized Rats

<table>
<thead>
<tr>
<th>Stage</th>
<th>NE, 100 ng/100 g</th>
<th>NE, 200 ng/100 g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-sham</td>
<td>4.7 ± 0.5</td>
<td>3.5 ± 0.8</td>
</tr>
<tr>
<td>Control-SAD</td>
<td>0.5 ± 0.5*</td>
<td>0.5 ± 0.08*</td>
</tr>
<tr>
<td>DOCA-sham</td>
<td>1.6 ± 0.4*†</td>
<td>1.3 ± 0.1*</td>
</tr>
<tr>
<td>DOCA-SAD</td>
<td>0.5 ± 0.2*</td>
<td>0.3 ± 0.1‡</td>
</tr>
<tr>
<td>F ratio</td>
<td>30.4</td>
<td>12.5</td>
</tr>
<tr>
<td>Prehypertensive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control-sham</td>
<td>8.9 ± 1.2</td>
<td>8.5 ± 0.8</td>
</tr>
<tr>
<td>Control-SAD</td>
<td>0.7 ± 0.1*‡</td>
<td>0.7 ± 0.1*‡</td>
</tr>
<tr>
<td>DOCA-sham</td>
<td>3.9 ± 0.7*</td>
<td>3.2 ± 0.7*</td>
</tr>
<tr>
<td>DOCA-SAD</td>
<td>0.9 ± 0.1*</td>
<td>0.6 ± 0.1‡</td>
</tr>
<tr>
<td>F ratio</td>
<td>28.1</td>
<td>51.3</td>
</tr>
</tbody>
</table>

Values indicate mean changes ± SEM from basal heart rate. NE = norepinephrine; SAD = sinoaortic denervation; sham = sham operation.

*p < 0.01, compared with values in control-sham rats.

Table 5. Sympathetic Nerve Activity Response to Intravenous Injection of Norepinephrine in Urethane-Anesthetized Rats

<table>
<thead>
<tr>
<th>Stage</th>
<th>SNA (spikes/sec/mm Hg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertensive</td>
<td></td>
</tr>
<tr>
<td>Control-sham</td>
<td>0.9 ± 0.17</td>
</tr>
<tr>
<td>Control-SAD</td>
<td>0.2 ± 0.03*</td>
</tr>
<tr>
<td>DOCA-sham</td>
<td>0.3 ± 0.07*</td>
</tr>
<tr>
<td>DOCA-SAD</td>
<td>0.2 ± 0.05*</td>
</tr>
<tr>
<td>F ratio</td>
<td>14.0</td>
</tr>
<tr>
<td>Prehypertensive</td>
<td></td>
</tr>
<tr>
<td>Control-sham</td>
<td>1.8 ± 0.33</td>
</tr>
<tr>
<td>Control-SAD</td>
<td>0.1 ± 0.021‡</td>
</tr>
<tr>
<td>DOCA-sham</td>
<td>0.2 ± 0.03</td>
</tr>
<tr>
<td>DOCA-SAD</td>
<td>0.2 ± 0.04‡</td>
</tr>
<tr>
<td>F ratio</td>
<td>5.8</td>
</tr>
</tbody>
</table>

Values indicate mean changes ± SEM from basal sympathetic nerve activity. SNA = sympathetic nerve activity; NE = norepinephrine; SAD = sinoaortic denervation; sham = sham operation.

*p < 0.01, †p < 0.05, compared with values in control-sham rats.

*p < 0.05, †p < 0.01, compared with values in DOCA-sham rats.
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