Sympathetic and Pressor Hyperresponsiveness to Intracisternal Injections of Hypertonic NaCl in DOCA Hypertensive Rats

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Blood pressure and sympathetic nerve activity were recorded before and after intracisternal injections of hypertonic NaCl solution in urethane anesthetized normotensive and deoxycorticosterone acetate (DOCA) hypertensive rats. Dose dependent pressor effects were recorded by intracisternal injections using normotensive Wistar rats. And the early phase of responses which were significantly depressed by blocking α-adrenergic receptors with phentolamine, accompanied by increased frequency of sympathetic nerve firing. Pressor responses and acceleration of the rate of sympathetic nerve firing produced by intracisternal injections of hypertonic NaCl were appreciably larger in DOCA hypertensives whose basal sympathetic nerve activity was elevated significantly than in normotensive rats. Pressor responses to intravenous injection of norepinephrine were also augmented, but responses to intracisternal injection were augmented more than those to norepinephrine injection. These findings suggest that sodium sensitive site which connects to pressor systems supposedly located around lower brain stem could be hypersensitive and eventually contribute to peripheral sympathetic hyperactivity in DOCA hypertension.

That sodium administrations into cerebrospinal fluid elevate blood pressure have been reported in awake and anesthetized several species. Also, it was found recently that intracisternal injections of hypertonic NaCl elicit enhanced pressor responses in anesthetized spontaneously hypertensive rat (SHR). However, the underlying mechanisms have not yet been clarified. In present studies, to elucidate the central effects of sodium administrations on peripheral output of sympathetic nerve activity and systemic blood pressure, we recorded sympathetic nerve activity and carotid pressure in urethane anesthetized normotensive and DOCA hypertensive rats before and after injecting hypertonic NaCl solution into cisterna magna.

MATERIALS AND METHODS
Female albino Wister rats weighing 200–250 g were used. DOCA hypertensive rats were made by subcutaneous injections of a suspension of deoxycorticosterone acetate 5 mg, twice a week and giving 1% NaCl solution ad libitum for 4 weeks after removing right kidney using male Sprague Dawley rat weighing 90–100 g. Unilateral nephrectomized rats were used as sham operated controls. All rats were anesthetized with urethane (1.2 g/kg). Blood pressure was recorded by connecting cannula inserted into the

Key Words:
DOCA hypertensive rat
Intracisternal injection
NaCl
Blood pressure
Sympathetic nerve activity

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left common carotid artery to a strain gauge transducer (MPV-290, Sanei Sokki). Heart rate was recorded simultaneously on another channel of the recorder counting it from the phasic pressure signals by a heart rate meter (Sanei Sokki). Drugs were injected into teflon tube cannulas inserted in the jugular veins.

Intracisternal injections were done using the rat mounted on the stereotaxic apparatus (David Kopf); Atolanto occipital membranes were exposed by dissecting the dorsal neck muscles and cisterna magna was punctured with 27 gauge needles connected through silastic tubing to a 50 μl microsyringe (Terumo). The needle was fixed to surrounding tissues with glue (Alon alpha, Konishi), when rats were placed on supine position for recording sympathetic nerve activity. Before the end of each experiment, methylene blue solution was injected to verify the needle position (methylene blue stained only ventral and dorsal brain stem, but not ventral surfaces).

Sympathetic nerve activity was recorded as described before6 The abdominal plexus was exposed, and a bipolar stainless steel electrode was placed on the major nerve bundles immediately below the celiac ganglion. Nerve and electrode tips were immersed in vegetable oil to reduce tissue drying. Spike potentials were amplified (Glass P15 AC amplifier) and monitored on a storage oscilloscope (Kikusui 5516 ST). To reduce noise during these recordings, spontaneous respiration was abolished by paralyzing skeletal muscles with decamethonium bromide (2 mg/kg) and connecting the rats to respirator ventilated with room air. Analog signal for aortic pressure and nerve activity were recorded continuously on magnetic tape (TEAC R210B).

To quantify the nerve activity, original analog signals were played back from the tape into inkless writing pen recorder and simultaneously fed into an spike counter (PSE 332P, Biomedical System), whose output was recorded separately as a histogram on the recorder and printed out as digitals. The low level control of window discriminator was routinely set to filter back ground noise persisting after crusing nerve bundle. Two drugs were injected through the jugular venous catheters: phentolamine mesylate (Regitine), 5 mg/kg and norepinephrine, 50, 100 and 200 ng/100 g, respectively. Data (average ± SEM) from rat groups were analyzed using Student's
TABLE 1  SYMPATHETIC NERVE ACTIVITY FOLLOWING INTRACISTERNAL INJECTION OF 10% NaCl SOLUTION IN URETHANE ANESTHETIZED RATS

<table>
<thead>
<tr>
<th>Rat group</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham-operated</td>
<td>20 ± 4</td>
<td>34 ± 4</td>
<td>48 ± 15</td>
<td>30 ± 7</td>
<td>33 ± 8</td>
</tr>
<tr>
<td>DOCA hypertensive</td>
<td>49 ± 10**</td>
<td>77 ± 14*</td>
<td>64 ± 24**</td>
<td>65 ± 17</td>
<td>37 ± 18</td>
</tr>
</tbody>
</table>

Average ± SEM (spikes/5 sec) of changes from baseline levels. * p < 0.02, ** p < 0.01

Fig.3. Pressor responses to injected norepinephrine (average ± SEM) in sham operated (open bars, n = 8) and DOCA hypertensive (striped bars, n = 5). Star marks indicate significant differences (p < 0.05).

t-test for comparing means of independent samples and differences at 5% level (p < 0.05) were considered significant.

RESULTS

Intracisternal injections of various hypertonic NaCl solutions produced dose dependent pressor responses in normotensive rats (0.9%: 3 ± 2, 2.5%: 7 ± 6, 5%: 20 ± 6, 10%: 44 ± 6 mmHg 3 min after intracisternal injection). These pressor responses reached their peak about 3 to 5 min after the injection and then gradually declined, but after 20 min blood pressure level was still over the baseline. In this pressor response, the early phase was significantly inhibited by the pre-treatment with phentolamine. In support of this fact, sympathetic nerve activity increased after the injections (Fig.1A). Adrenalectomy did not affect this pressor responses. The late phase of the response was significantly reduced in hypophysectomized rats.

Pressor Effect of Intracisternal Injections of Hypertonic NaCl in DOCA Hypertensive Rats

Basal mean aortic pressure (mmHg ± SE), which averaged 110 ± 8 in DOCA hypertensive rats, was higher (p < 0.02) during urethane anesthesia than 85 ± 4 mmHg in sham operated controls. Intracisternal injections of 10% NaCl solution increased aortic pressure, which showed its peak 4 min after the injection in both DOCA hypertensive and sham operated rats (Fig.2). Pressor responsiveness was appreciably greater in DOCA hypertensive than in normotensive rats.

Sympathetic Nerve Activity following Intracisternal Injections of Hypertonic Solution

Integrated potentials of baseline level in the abdominal sympathetic nerves were significantly higher (p < 0.01) in DOCA hypertensives than in controls, and averaged base lines were 57 ± 4 and 36 ± 3 (spikes/5 sec ± SE) respectively. Nerve activity increased gradually following intracisternal injections and their maximum increases were produced at 2 min after the injection in DOCA hypertensive and at 3 min in sham operated rats (Fig.1). Acceleration in firing rates during 3 min was more pronounced in DOCA hypertensive than in normotensive rats (Table I).

Blood Pressure Responsiveness to Intravenously Injected Norepinephrine and Phentolamine

Aside from the pressor effects of the intracisternal injections of NaCl, similar responses induced by injecting graded doses of norepinephrine were also compared. Increases in mean aortic pressure were larger in DOCA hypertensive than in sham operated normotensive rats (Fig.3). However, pressor responses to intracisternal injection of 10% NaCl solution tended to be
more strongly enhanced than responses to norepinephrine injection. When α adrenergic receptors were later blocked with phentolamine, mean aortic pressure fell more in DOCA hypertensive (-72 ± 7) than in normotensive (-50 ± 4 mmHg) rats (P < 0.02). These results suggest that sympathetic vasomotor tone plays an important role to maintain high blood pressure in DOCA hypertension.

**DISCUSSION**

Intracisternal injections of hypertonic NaCl solution produced pressor responses accompanied by corresponding sympathetic hyperactivity in the early phase of the responses. These findings suggest that brain has NaCl sensitive site which leads to sympathetic hyperactivity. Probably, sodium is really active ion to produce pressor responses, because pressor effects were obtained with various sodium salt as previously reported by Wei and Wu. The sodium sensitive site may be located around the lower brain stem, since methylene blue injected at the end of each experiment did not stain any ventricles, and this site may be different from those in the third ventricles which were described in several species of animals. In DOCA hypertensive rats, pressor effects of intracisternal injections of hypertonic NaCl were significantly larger than in controls in this study. Enhanced pressor effects induced by administration of hypertonic NaCl into the central spinal fluid were also found in SHR and Dahl salt hypertensive rats. But in our results this enhanced pressor effects were noted only in the early phase of the pressor responses. Therefore, it seems logical to assume that this enhancement of pressor responsiveness was due mainly to augmented sympathetic hyperactivity. But this can not be the sole mechanisms involved, because pressor responses to injected norepinephrine were also augmented thereby implying that vascular sensitivity had also been increased. Recently, the evidences showing that sympathetic nervous system responsible to development and maintenance of hypertension have been accumulated in SHR, DOCA hypertensive and salt induced hypertensive rats. In this study, sympathetic hyperactivity in DOCA hypertensive rats, which we reported previously is again confirmed by direct recording of sympathetic nerve potentials and by augmented vasodepressor responses to α adrenergic blockade with phentolamine.

· These findings may support the idea that basal sympathetic hyperactivity could be partly due to the hypersensitivity to sodium in the brain in DOCA hypertensive rats.

**REFERENCES**


*Japanese Circulation Journal Vol. 45, September 1981*