CENTRAL AND PERIPHERAL MECHANISMS OF THE ENHANCED HYPERTENSION FOLLOWING LONG-TERM SALT LOADING IN SPONTANEOUSLY HYPERTENSIVE RATS.

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We evaluated whether or not increased sodium (Na) concentrations of cerebrospinal fluid (CSF) and stimulated activities of brain renin-angiotensin system (RAS) contribute to an enhanced hypertension by salt overload in spontaneously hypertensive rats (SHR). Long-term salt loading (1% NaCl solution as drinking fluid) accelerated the development of hypertension in SHR, but did not alter the blood pressure (BP) in normotensive Wistar-Kyoto rats (WKY). CSF Na concentration was elevated in uninephrectomized (Nx) group as compared to that in control SHR, while in WKY CSF Na was not influenced by the treatment. A fall in BP by intravenous AVP antagonist or hexamethonium was greater in salt-loaded SHR than in controls. This hypotensive response to the combined blockade of AVP and SNS correlated with CSF Na in SHR but not in WKY. Plasma concentration of AVP and epinephrine tended to increase in relation to the degree of salt loading in SHR but not in WKY. Pressor responses to intracerebroventricular (ICV) angiotensin II (AII) and NaCl were greater in SHR than in WKY, although these responses were not influenced by chronic salt load in either SHR or WKY. The enhanced hypertensive action of ICV NaCl in SHR was abolished by pretreatment with ICV AII antagonist. Chronic saline drinking enhanced the depressor effect of ICV captopril in SHR but not in WKY. These observations suggest that salt overload in SHR may cause an elevated CSF Na concentration and an enhanced activity of brain RAS, which may increase activity of SNS and release of AVP, resulting in an enhanced development of hypertension.

LONG-TERM salt loading increases blood pressure (BP) in spontaneously hypertensive rats (SHR) but not in normotensive rats. Sympathetic nervous system (SNS) is enhanced in SHR but suppressed in normotensive controls following chronic salt overload. DOCA-salt hypertension in rats, which is known as a salt dependent hypertension, is attributed to the increased activity of SNS and stimulated release of vasopressin (AVP). Thus, it is likely that an enhanced hypertension induced by salt overload in SHR is derived from the hyperactivity of both SNS and AVP in a manner similar to that in DOCA-salt rats.

Intracerebroventricular (ICV) administrations of angiotensin II (AII) or hypertonic NaCl solution in rats stimulate activity of SNS and release of AVP, resulting in an increase in BP. Hypertonic NaCl injected ICV activates the central renin-angiotensin system (RAS) in rats.

Key words:
Salt, CSF, Vasopressin
Sympathetic nervous system
Brain renin-angiotensin system

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indicating an interrelation between cerebrospinal fluid (CSF) Na concentration and central RAS. Additionally, this brain RAS activity is enhanced in SHR, in comparison with in normotensive Wistar-Kyoto rats (WKY). These observations described above suggest that long-term salt over-load in SHR may accelerate the development of hypertension as a result of increased central sodium concentrations and enhanced activities of brain RAS which stimulates SNS and AVP release.

The purpose of the present study was to determine whether or not chronic salt overload in SHR could increase CSF sodium concentrations and activate central RAS, and also if SNS and AVP release could be enhanced in relation to these central mechanisms.

METHODS

Experiment 1: Male SHR or WKY of 5 weeks old were separated into 3 groups, (i) Uninephrectomy + 1% NaCl solution as drinking fluid (Nx group), (ii) Sham operation + 1% NaCl solution (S group), (iii) Sham operation + plain tap water (C group). All animals received a standard pellet chow (Na 250 mg/100 g, K 800 mg/100 g).

Systolic blood pressure (SBP) was recorded every week by tail-plethysmography before and during the treatment. Cerebrospinal fluid (CSF) of 100 µl was drawn from cisterna magna by 28 gauge needle puncturing the atlanto-occipital membrane, and blood of 0.3 ml from tail vein in order to measure sodium concentration on the 3rd and 7th week of salt loading.

After 7 weeks of treatment, catheters of PE10 polyethylene tubing were inserted into femoral artery and vein under light ether anesthesia. One day later, mean arterial pressure (MAP) and heart rate (HR) were recorded directly from the indwelling catheter on a polygraph (RM-6200, Nihon-Kohden, Japan) in a conscious and unrestrained state. After a period of at least 30 min for stabilization of MAP and HR, the vascular antagonist, AVP, d(CH$_2$)$_5$Tyr(Me)AVP (AVPA, 10 µg/kg) was injected intravenously. Twenty minutes after AVPA injection, hexamethonium (C$_6$, 30 mg/kg) was sequentially administered. The next day, with a continuous recording of MAP and HR, C$_6$ was firstly administered, and after 20 min AVPA was injected intravenously. The doses of AVPA and C$_6$ used in this experiment were shown, in a pilot study using SHR in a conscious state, to nearly abolish 20 to 30 mmHg rise in MAP induced by AVP (30 mU/kg iv) and 1, 1-dimethyl-4-phenyl-piperazinium iodide (30 µg/kg iv), respectively, for at least 60 min.

In another set of experiments, male SHR and WKY of 5 weeks old were similarly divided into 3 groups of Nx, S and C. After 7 weeks treatment, 3 different doses of norepinephrine (NE: 30, 100, 300 ng/kg), AVP (3, 10, 30 mU/kg) and angiotensin II (AI; 10, 30, 100 ng/kg) were injected intravenously with a continuous recording of MAP and HR in conscious and unrestrained rats cannulated 24 hours before. On the following day, 0.7 ml of blood was drawn from the indwelling arterial catheter to measure plasma catecholamine concentrations, and thereafter 1.2 ml of blood was drawn to assay plasma AVP.

Plasma level of catecholamines was analyzed by radioenzymatic methods (Upjohn company, USA), and that of AVP by radioimmunoassay (Mitsubishi-yuka Laboratory of Medical Science Co. Ltd., Japan). Serum sodium concentration was determined by flame photometry with Ciliatun as the internal standard (Hitachi Flame Photometer 205D, Japan).

Experiment 2: Male SHR and WKY of 5 weeks old were divided into 2 groups of (i) 1% NaCl solution as a drinking fluid (S group) and (ii) plain tap water (C group). SBP was recorded weekly by tail-plethysmography before and during treatment for 7 weeks.

During the 7th treatment week, under pentobarbital anesthesia (50 mg/kg ip), a cannula of 23 gauge was implanted into the lateral cerebroventricle using a stereotaxic apparatus with coordinates of 1.5 mm posterior from the anterior suture line, 2 mm lateral from the middle, and 5 mm deep from the skull surface. The following day, AII of 100 ng/kg was injected into the lateral ventricle of all rats in a conscious state. Only rats that showed a drinking behaviour in response to ICV AII were used in the following experiments.

One day after the cannula implantation into cerebroventricle, catheters of PE10 tubing were similarly inserted into femoral artery and vein under light ether anesthesia. One to 3 days after the vascular surgery, the following experiments were performed on different days with a continuous recording of MAP and HR. On the first experiment day, AII (0.3, 3.0, 30 ng/kg) and hypertonic saline (0.2, 0.3, 0.5M) were given ICV at a volume of 10 µl/kg. On the following day, 0.3 ml of blood was taken to measure plasma renin concentration (PRC) from the indwelling

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TABLE I  SODIUM (Na) CONCENTRATION OF CEREBROSPINAL FLUID (CSF) AND SERUM, AND SYSTOLIC BLOOD PRESSURE (SBP) AND MEAN ARTERIAL PRESSURE (MAP) IN SPONTANEOUSLY HYPERTENSIVE RATS (SHR) AND NORMOTENSIVE Wistar-Kyoto Rats (WKY)

<table>
<thead>
<tr>
<th></th>
<th>CSF Na</th>
<th>Serum Na</th>
<th>SBP</th>
<th>MAP</th>
</tr>
</thead>
<tbody>
<tr>
<td>3 Weeks</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SHR-Nx</td>
<td>151.1 ± 0.4</td>
<td>141.7 ± 0.8</td>
<td>179 ± 7</td>
<td></td>
</tr>
<tr>
<td>SHR-S</td>
<td>151.1 ± 0.4</td>
<td>141.0 ± 0.6</td>
<td>181 ± 6</td>
<td></td>
</tr>
<tr>
<td>SHR-C</td>
<td>151.8 ± 0.3</td>
<td>140.3 ± 0.3</td>
<td>175 ± 6</td>
<td></td>
</tr>
<tr>
<td>WKY-Nx</td>
<td>151.6 ± 0.3</td>
<td>140.5 ± 0.6</td>
<td>122 ± 2</td>
<td></td>
</tr>
<tr>
<td>WKY-S</td>
<td>151.6 ± 1.0</td>
<td>139.7 ± 0.8</td>
<td>123 ± 3</td>
<td></td>
</tr>
<tr>
<td>WKY-C</td>
<td>151.0 ± 1.1</td>
<td>141.2 ± 0.8</td>
<td>121 ± 1</td>
<td></td>
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<tr>
<td>7 Weeks</td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>SHR-Nx</td>
<td>152.8 ± 0.3**</td>
<td>141.0 ± 0.7</td>
<td>223 ± 2**</td>
<td>203 ± 4**</td>
</tr>
<tr>
<td>SHR-S</td>
<td>151.5 ± 0.2</td>
<td>141.6 ± 0.9</td>
<td>213 ± 3**</td>
<td>182 ± 3</td>
</tr>
<tr>
<td>SHR-C</td>
<td>151.4 ± 0.3</td>
<td>140.6 ± 0.5</td>
<td>203 ± 2</td>
<td>174 ± 2</td>
</tr>
<tr>
<td>WKY-Nx</td>
<td>150.5 ± 0.4</td>
<td>140.0 ± 0.5</td>
<td>127 ± 1</td>
<td>127 ± 1</td>
</tr>
<tr>
<td>WKY-S</td>
<td>150.0 ± 0.4</td>
<td>142.7 ± 0.6</td>
<td>127 ± 1</td>
<td>129 ± 2</td>
</tr>
<tr>
<td>WKY-C</td>
<td>150.9 ± 0.3</td>
<td>142.8 ± 2.5</td>
<td>127 ± 1</td>
<td>129 ± 2</td>
</tr>
</tbody>
</table>


t = uninephrectomy + 1% NaCl drinking; S = sham operation + 1% NaCl drinking; C = sham operation + plain tap water drinking. The numbers of rats used for each experiment are 6 to 14. **p < 0.01, in comparison with the corresponding control group.

arterial catheter. PRC was determined by radio-immunoassay for generated angiotensin I (AI) incubated with 80 μl of rat angiotensinogen which was prepared according to the method of Haas et al.3 Thereafter, captopril of 300 μg/kg was injected intravenously. The next day, captopril (300 μg/kg) was injected ICV, and MAP and HR were recorded for 3 hours.

**Experiment 3:** Male SHR and WKY of 4 to 6 months old were anesthetized with pentobarbital. A 23 gauge cannula was similarly implanted into the lateral ventricle, and the correct placement of the cannula was verified by AI-induced drinking behaviour a few days after the surgery. The following day, PE10 tubings were cannulated into the animals' femoral artery and vein. A few days after the cannulation, physiological and hypertonic NaCl solutions (0.15, 0.2, 0.25, 0.3, 0.5M; 10 μl/kg) were injected ICV. All doses except 0.15M saline were then repeated after the administration of the AI analogue, 1-Sar, 8-Ileu-AII (Ala, 100 μg/kg) into the lateral ventricle. Preliminary experiments showed that this dose of AIIA substantially abolished the pressor response to ICV AII 100 ng/kg for at least 3 hours.

All values given are the mean ± SEM. Analysis of differences between groups was accomplished by 2 way analysis of variance (ANOVA) and t-test. Some of dose-dependent lines were analyzed by analysis of co-variance. Linear correlation and regression coefficients were calculated. Results were considered significant when p < 0.05.

**RESULTS**

**Experiment 1:** Salt loading with or without uninephrectomy did not alter SBP in SHR up to the 3rd treatment week, but thereafter it gradually increased SBP which was significantly higher in Nx or S group than in the C-SHR at the 7th week of treatment. In WKY, SBP was not influenced by the treatment for 7 weeks (Table I). The MAP directly recorded from indwelling catheter in a conscious state also showed a significant difference between Nx and C-SHR.

The CSF Na concentration was not altered by salt overload for 3 weeks in either SHR or WKY, as shown in Table I. On the 7th treatment week, however, salt intake with uninephrectomy in SHR significantly raised CSF Na as compared to the controls, while in WKY CSF Na on the
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7th week did not differ among the 3 groups. In contrast to the results of CSF Na, serum Na concentration was not altered by the treatment for 3 or 7 weeks in either SHR or WKY. In SHR, CSF Na concentration was significantly correlated with MAP recorded from indwelling catheter on the 7th treatment week (r = 0.38, p < 0.02).

The fall in MAP induced by intravenous AVPA was greater in Nx-SHR than that in control (F = 4.3, p < 0.05). The subsequent injection of C6 also resulted in enhanced depressor response in Nx-SHR as compared to that in either S or C group (F = 4.7, p < 0.05, Nx vs S; F = 9.1, p < 0.01,Nx vs C), resulting in an abolition of difference in MAP among 3 groups of SHR. Similarly, the reversed order of blocking agent injection caused greater depressor responses in Nx-SHR than in control SHR (p < 0.05). The fall in MAP induced by combined blockade of AVP and SNS was greater in Nx than that in either S or C group of SHR (F = 5.5, p < 0.05, Nx vs S; F = 11.4, p < 0.005, Nx vs C). In WKY, however, AVPA or C6 lowered MAP to a similar degree in the 3 different groups. The amplitude of decrease of MAP by combined inhibition by AVPA + C6 in SHR significantly correlated with CSF Na concentration on the 7th treatment week (r = 0.41, p < 0.02).

Intravenous injections of AVP, NE or AII induced a dose-dependent pressor responses in either group of SHR or WKY. The dose-related pressor and bradycardic responses to these vasoconstrictive agents were similar among 3 groups of SHR or WKY. Plasma AVP level tended to increase in parallel with the degree of the salt loading in SHR, and was significantly higher in Nx than that in control group (p < 0.05). Epinephrine (E) also showed a tendency to increase in response to salt loading in SHR, though its difference between groups was not significant. In contrast, the concentrations of AVP and E were not altered by the treatment in WKY. NE level was not influenced by the salt loading in either SHR or WKY. Plasma AVP, E and NE were consistently higher in SHR than that in the corresponding group of WKY (p < 0.05).

Experiment 2: Saline drinking without uninephrectomy resulted in a gradual but consistent increase in SBP in SHR. The difference between groups was significant on the 6th and 7th treatment week (p < 0.01). In contrast, SBP was not altered by the salt loading in WKY.

Both AII and hypertonic saline injected ICV produced a dose-dependent pressor action in both SHR and WKY groups. SHR produced significantly greater pressor responses to ICV NaCl than did WKY (F = 8.8, p < 0.005). The slope of the dose response line of ICV AII was steeper in SHR than in WKY (F = 7.2, p < 0.01). However, neither pressor response to AII nor hypertonic NaCl was influenced by salt loading in SHR or WKY.

PRC of blood taken through the indwelling arterial catheter was significantly suppressed by salt treatment in both SHR and WKY (p < 0.05). Similarly, the hypotensive response to IV captopril was diminished by the chronic saline drinking in SHR (F = 17.2, p < 0.001).

ICV captopril lowered MAP by approximately 10 mmHg in salt-loaded SHR, while the non-treated SHR did not respond to ICV captopril. The fall in MAP was much greater 35 min after ICV injection in salt-loaded group than that in control SHR (F = 27.1, p < 0.001). In contrast with the findings of SHR, the salt overload did not induce any hypotensive response to ICV captopril in WKY of either group.

Experiment 3: ICV NaCl caused a dose-dependent pressor action, which was significantly greater in SHR than in WKY (F = 4.5, p < 0.05). Doses above 0.2 M NaCl caused significant increases in MAP compared with the physiological NaCl solution in SHR by paired t-test (p < 0.05). The WKY rats did not respond to ICV NaCl in doses up to 0.3 M. The pressor effect of ICV NaCl was partially suppressed by ICV AIIA in SHR (F = 6.9, p < 0.025), although the same dose of ICV AIIA had no effect on the pressor action of hypertonic NaCl in WKY. The increase in MAP induced by ICV NaCl was similar in SHR and WKY after treatment with ICV AIIA.

DISCUSSION

In the present study, chronic salt loading combined with uninephrectomy caused an increase in CSF Na concentration in SHR. Salt treatment without uninephrectomy enhanced a depressor effect of ICV captopril, indicating that salt loading may increase BP as a result of an increased activity of brain RAS in SHR. Together with the findings that pressor responses to ICV AII and hypertonic NaCl were greater in SHR than in WKY, it is likely that, in SHR, increases in CSF Na concentration and in activity of central RAS may be main contributing factors to the enhanced development of hypertension following long-term salt loading. This is in agree-
ment with the observations that CSF Na correlated with BP in SHR during the salt treatment and that blockade of brain RAS abolished a difference in BP between treated and control SHR.

The hypersensitivity to ICV NaCl in SHR may be attributed to the accelerated activity of endogenous brain AII, since blockade by ICV AIIA abolished the hyperreactivity of SHR to ICV hypertonic NaCl and resulted in a similar pressor action of ICV NaCl in both SHR and WKY. The hyperactivity of brain RAS in SHR was shown by the enhanced pressor responses to ICV AII and activated depressor responses to ICV captopril.

Increased activities of SNS and AVP system in SHR induced by salt treatment were suggested by the findings that the hypotensive effect of AVPA or C6 was significantly greater in salt loaded groups than in controls and that plasma AVP and E tended to increase in response to the salt loading. Since an increase in CSF Na by ICV NaCl is known to elevate BP by activating SNS and AVP release9,10 the present observation, that the fall in MAP by the combined blockade of AVP and SNS correlated with CSF Na, suggest that an increased CSF Na concentration in Nx SHR may stimulate both SNS and AVP release and raise BP. This hypertensive action of an increased CSF Na concentration may be partly attributed to an activated brain RAS, because AIIA diminished pressor responses to ICV NaCl in SHR.

An increased vascular responsiveness and a suppressed activity of barofunction may not be responsible for the enhanced hypertension by the salt loading, since the treatment did not alter the dose-related pressor and bradycardic responses to AII, NE and AVP in SHR.

The peripheral RAS was suppressed in response to salt load as indicated by reduction in both PRC and depressor effect of IV captopril. This observation, together with the enhancement of hypotensive effect of ICV captopril in SHR induced by salt loading, suggest that brain RAS is independent of the peripheral system.

In DOCA-salt hypertensive rats, SNS and AVP systems are augmented, and this enhanced activity of both pressor systems contributes to the increase in BP7. Although the peripheral mechanisms in increasing BP are similar between DOCA rats and salt loaded SHR in the present study, CSF Na is not altered in DOCA-salt rats14 but was elevated in SHR. Other central mecha-

11. STAMLER JF, PHILLIPS MI: Attenuation of the central hypertonic NaCl pressor response by angio-

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REFERENCES


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