The Role of Chloride on Deoxycorticosterone Acetate-Salt Hypertension

TAKAAKI MOTOYAMA, M.D., HIROSHI SANO, M.D., HIROSHI SUZUKI, M.D.
KEIZO KAWAGUCHI, M.D., HISASHI FUKUZAKI, M.D., JUNJI YAMANISHI, M.D.*
YUTAKA FURUTA, M.D*, TAKEHIRO OMATSU, M.D**
AND KOMEI SAITO, M.D**

Selective sodium loading attenuated the development of hypertension in the deoxycorticosterone acetate (DOCA) treated rat. The DOCA treated rat fed a diet equimolar in sodium to a 7% sodium chloride diet and in chloride to a standard diet, differed in various parameters from the DOCA treated rat fed a 7% sodium chloride diet: it had higher sodium concentration in both erythrocytes and muscles, a higher erythrocyte ouabain sensitive $^{22}$Na efflux rate constant (Kos), and a lower norepinephrine turnover rate in the heart and the spleen.

These results suggest that the suppressed sympathetic nervous system activity and the activated cell membrane sodium pump contribute in part to the mechanism for the suppression of the development of hypertension in the DOCA-selective sodium loaded rat.

THE mechanism by which a high dietary sodium chloride intake produces hypertension in the susceptible host has not been defined, although epidemiological surveys$^{1-4}$ and numerous clinical and experimental studies$^{5-13}$ have established the fact that the increased consumption of salt promotes the development of hypertension. Recent evidences$^{14-18}$ suggest an important role of chloride for the development of hypertension in the Dahl salt-sensitive rat and the deoxycorticosterone acetate (DOCA)-salt rat.

The present study was performed to elucidate the effects of selective sodium loading on blood pressure and to evaluate the roles of chloride in sympathetic nervous system and cell membrane sodium transport in the development of hypertension in the DOCA-salt hypertensive rat.

MATERIALS AND METHODS
Male Wistar rats weighing 180 to 220g were uninephrectomized and individually housed in metabolic cages. The rats were divided into three groups. The first group (n = 14) remained on a standard laboratory rat chow diet (the Control group); the second group (n = 20) was fed a 7% sodium chloride diet by weight (the DOCA-NaCl group) and the third group (n = 16) received a diet equimolar in sodium to the 7% sodium chloride diet and in chloride to the standard diet (an NaAA diet) (the DOCA-NaAA group). To prepare the NaAA diet, the following substances were added to 100g of the standard diet by the method of Whitescarver et al$^{16}$ with a slight modification: 5 mEq of sodium phosphate, 15 mEq of sodium bicarbonate, 40 mEq of monosodium aspartate and 40 mEq of monosodium glutamate. Rats in the DOCA-NaCl and the DOCA-NaAA groups were injected subcutaneously 30 mg/kg DOCA every week. All of rats

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The First Department of Internal Medicine, Kobe University School of Medicine; *Internal Medicine, Kasai Hospital; **Internal Medicine, Hidaka Hospital; Japan
Mailing address: Takaaki Motoyama, M.D., The First Department of Internal Medicine, Kobe University School of Medicine, 7-5-2 Kusunoki-cho, Chuo-ku, Kobe 650, Japan

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TABLE I SYSTOLIC BLOOD PRESSURE (mmHg), HEART RATE (/min) AND BODY WEIGHT (g) AT THE ENTRY AND THE END OF THE STUDY

<table>
<thead>
<tr>
<th>Group</th>
<th>Blood Pressure</th>
<th>Heart Rate</th>
<th>Body Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0W</td>
<td>4W</td>
<td>0W</td>
</tr>
<tr>
<td>Control</td>
<td>123 ± 18</td>
<td>132 ± 4</td>
<td>433 ± 24</td>
</tr>
<tr>
<td>DOCA-NaCl</td>
<td>116 ± 17</td>
<td>205 ± 17*+</td>
<td>417 ± 39</td>
</tr>
<tr>
<td>DOCA-NaAA</td>
<td>120 ± 14</td>
<td>169 ± 8***</td>
<td>444 ± 31</td>
</tr>
</tbody>
</table>

***p < 0.001 vs Control
†† p < 0.01, ††† p < 0.001 DOCA-NaCl vs NaAA

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Fig. 1. Systolic blood pressure during the study in the Control (○), the DOCA-NaCl (▲) and the DOCA-NaAA (●) groups.

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Concentrations, intramuscular and intraaortic sodium concentrations after nitric acid digestion, erythrocyte membrane sodium pump, and norepinephrine concentrations in the heart and the spleen. Intraerythrocyte sodium, potassium and magnesium concentrations were measured by the method of Kaya et al.²⁹ with a slight modification. Erythrocyte membrane sodium pump was measured as erythrocyte ouabain sensitive ²²Na efflux rate constant (Kos) by the method of Walter et al.²⁹ with a slight modification. Both of these techniques have been described previously.²¹ Intraerythrocyte chloride concentration was calculated as blood chloride content minus plasma chloride content.

Rats in the second course (n = 7 in the Control group, n = 10 in the DOCA-NaCl group, n = 8 in the DOCA-NaAA group) were killed by decapitation to measure norepinephrine concentrations in the heart and the spleen 4 hours after inhibition of norepinephrine synthesis by intraperitoneal injection of DL-α-methyl-p-tyrosine (300 mg/kg). Norepinephrine turnover rate constants in the heart and the spleen were calculated by the regression analysis test with the data of norepinephrine concentrations in rats of the first and the second courses.

(Analytic Methods)
Sodium and potassium concentrations were measured with a flame photometer, calcium and magnesium were determined with an atomic absorption spectrophotometry, and chloride was measured with a chloridometer. Tissue norepinephrine was extracted, separated by high-performance liquid chromatography, and determined by the trihydroxyindole method.

(Statistical Methods)
Comparison of the data between groups of

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subjects was made with Student's t test. Correlation of the data was sought using a least squares fit linear regression analysis. Values were expressed as means ± standard deviation. A p value less than 0.05 was considered significant.

RESULTS

Table I shows systolic blood pressure, heart rate and body weight in each group at the entry and the end of the study. Those values were similar for all three groups at the entry of the study. For the duration of the study, blood pressure of the DOCA-NaCl group was higher than that of the DOCA-NaAA group, which was higher than that of the Control group during the last two weeks of the study (Fig. 1). Heart rate of the DOCA-NaAA group was lower than those of the Control and the DOCA-NaCl groups during the last two weeks of the study. Body weight between the DOCA-NaCl and the DOCA-NaAA groups gave no significant difference throughout the study. There was also no difference in dietary intake among all three groups throughout the study, so both the DOCA-NaCl and the DOCA-NaAA groups were similar in dietary sodium intake and both the Control and the DOCA-NaAA groups were similar in dietary chloride intake.

Table II presents urinary volume and electro-
lytes excretion in each group at the end of the study. Urine volume and water intake of the DOCA-NaCl group were slightly greater than in the DOCA-NaAA group, which were, in turn, much greater than those of the Control group. Urinary sodium and calcium excretion of two DOCA treated groups, which were not significantly different from each other, were higher than those of the Control group. Higher urinary chloride excretion was noted in the DOCA-NaCl group as compared with the Control and the DOCA-NaAA group.

Electrolyte concentrations in the plasma, the muscle and the aorta of each group after the study are given in Table III. There was no significant group difference in plasma sodium concentration. Lower plasma chloride concentration was noted in the DOCA-NaAA group as compared with the Control and the DOCA-NaCl groups, however, no significant difference was found between the latter two groups. Plasma magnesium concentration of the DOCA-NaAA group was lower than those of the Control and the DOCA-NaCl groups. Two DOCA treated groups were similar in plasma calcium concentration. Sodium concentrations in both the muscle and the aorta of the DOCA-NaAA group were greater than those of the Control group although any difference in intracellular sodium concentrations either between the DOCA-NaAA and the DOCA-

NaCl groups or between the DOCA-NaCl and the Control groups was not significant.

Results of measurements for Kos and intraerythrocyte electrolyte concentrations in each group after the study are presented in Table IV. Intraerythrocyte sodium and potassium concentrations of the DOCA-NaAA group were higher than those of the DOCA-NaCl group, which were, in turn, higher than those of the Control group, although difference in intraerythrocyte potassium concentrations between the DOCA-NaCl and the Control groups was not significant. Intraerythrocyte chloride concentration of the DOCA-NaAA group tended to be lower than that of the DOCA-NaCl group, which tended to be lower than that of the Control group, but only the difference between the DOCA-NaAA and the Control groups was significant. There was greater intraerythrocyte magnesium concentration in the DOCA-NaAA group as compared with the Control and the DOCA-NaCl groups. Furthermore, Kos of the DOCA-NaAA group was higher than that of the DOCA-NaCl group, which was higher than that of the Control group.

Figures 2, 3 and 4 give the relationships among

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Kos, intraerythrocyte electrolytes and blood pressure. As shown in Fig. 2, Kos is inversely proportional to systolic blood pressure in the DOCA-NaCl and the DOCA-NaAA groups. There is also a positive correlation between intraerythrocyte magnesium concentration and Kos, and an inverse correlation between intraerythrocyte magnesium and chloride concentrations. (Figs. 3 and 4)

Data of norepinephrine concentrations and turnover rate constants in the heart and the spleen in each group after the study are summarized in Table V. Although norepinephrine concentrations in the heart and the spleen were similar for the DOCA-NaAA and the DOCA-NaCl groups, norepinephrine turnover rates in the heart and the spleen of the DOCA-NaAA group were less than those of the DOCA-NaCl group, and similar to those of the Control group.

DISCUSSION

DOCA treated uninephrectomized male Wistar rats fed a high sodium chloride diet showed an elevation of blood pressure within one week of DOCA administration. In contrast, a high sodium and normal chloride diet with assorted anions containing an equivalent amount of sodium to the high sodium chloride diet attenuated the elevation of blood pressure.

Kurz T.W. et al. reported that the increase in mean arterial pressure of male Sprague-Dawley rats given DOCA and sodium chloride was significantly greater than in rats given DOCA and equimolar amounts of sodium bicarbonate, sodium ascorbate, or a combination of sodium bicarbonate and sodium ascorbate. In the latter rats the severity of hypokalemia and metabolic alkalosis was greater than in rats given DOCA and sodium chloride. It was reported that under certain circumstances, potassium depletion was found to reduce blood pressure. The mechanism resulting in a lower blood pressure in rats given sodium bicarbonate, or sodium ascorbate, or both, compared to rats given sodium chloride has not been clarified. In the study by White-scaver S.A. et al. Dahl salt-sensitive rats were fed a diet containing a normal or high concentration of sodium chloride or a high concentration of sodium, provided as mixture of sodium bicarbonate, phosphate, aspartate, glutamate and glycinate. In the group of rats receiving a diet with a high concentration of sodium chloride,

<table>
<thead>
<tr>
<th>TABLE V NOREPINEPHRINE (NE) CONCENTRATIONS (ng/g) AND TURNOVER RATE CONSTANTS (/h) IN THE HEART AND THE Spleen AFTER THE STUDY</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>DOCA-NaCl</td>
</tr>
<tr>
<td>DOCA-NaAA</td>
</tr>
</tbody>
</table>

*p < 0.05, **p < 0.01, ***p < 0.001 vs Control
†p < 0.05, ††p < 0.01, DOCA-NaCl vs NaAA

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systolic blood pressure was higher than in the other two groups. A comparison between the two groups of rats with high concentrations of sodium chloride and sodium with normal concentration of chloride showed no significant difference in plasma volume, arterial pH, plasma sodium, potassium, chloride or calcium concentration, plasma renin activity, plasma aldosterone concentration or renomedullary prostaglandin E2 production. In the rats given a diet with high concentration of sodium without chloride, the net sodium balance was greater, the net chloride balance was less and the muscle sodium content was greater than those in the other two groups. In their study, they concluded that there were implications for future studies on the mechanism by which the development of hypertension in Dahl salt-sensitive rat was dependent on the provision of sodium as sodium chloride. Passmore J.C. et al. did a similar study with another sodium chloride-dependent model rat of hypertension, the DOCA-salt hypertensive rat. They found no difference between net sodium or potassium balance or total carcass sodium or potassium content in the two DOCA treated groups. They also found that the higher blood pressure in the DOCA-salt treated rat was not associated with sodium, but with a more positive chloride balance, increased total carcass chloride content, an expanded extracellular fluid volume and increased renal vascular resistance. In order to examine the effect of dietary chloride loading on blood pressure, they fed the Dahl salt-sensitive rat a 1% sodium chloride diet, a 4% sodium chloride diet or a normal sodium and high chloride diet made equimolar in chloride to the high sodium chloride diet by adding glycine chloride. Consequently, the rats receiving a high sodium chloride diet had higher blood pressure than the other two groups which were not different from each other. In their studies, it was indicated that the combination of sodium with chloride was responsible for the development of hypertension in these salt-sensitive model rats and that an expanded extracellular fluid volume and increased renal vascular resistance might play an important role in the pathogenesis of the DOCA-salt hypertension.

In our study, there was not any difference in dietary sodium intake, urinary sodium excretion or plasma or intraaortic sodium concentration in two DOCA treated groups. Furthermore, we found higher sodium concentrations in the erythrocytes and the muscle of the DOCA-NaAA group than in the DOCA-NaCl group. These results indicate that the differences in the developments of hypertension in two DOCA treated rats is not associated with sodium retention, although the important role of sodium in the pathogenesis of hypertension and the relation of sodium retention to the development of hypertension is widely recognized. In the DOCA-NaAA group, dietary chloride intake, urinary chloride excretion and plasma chloride concentration were less than in the DOCA-NaCl group. Salt-dependent hypertension in the DOCA-NaCl rat, therefore, may be attributable to chloride.

The electroneutral sodium-hydrogen ion exchange pump at the lumen border of the renal tubule, is one of main mechanisms of hydrogen ion secretion and is generally accepted to be the principal process involved in urinary acidification. It has been proposed that this pump is dependent on a sodium concentration gradient across the luminal cell membrane and Na⁺ - K⁺ ATPase pump activity at the basolateral membrane. On the other hand, one of the main mechanisms responsible for the secretion of bicarbonate ions is a chloride-bicarbonate ion exchange system which acts in parallel to a sodium-hydrogen ion countertransport, which itself is dependent on chloride and not sodium. A high concentration of sodium and a normal concentration of chloride in the lumen of the renal tubule probably cause an acceleration of sodium-hydrogen ion exchange and sodium-potassium ion countertransport, due to the activation of Na⁺ - K⁺ ATPase, a decrease in intracellular hydrogen ion and an increase in intracellular bicarbonate ion. Although the increase in intracellular bicarbonate ion is partially reduced by secretion through the chloride-bicarbonate ion exchange system, the high sodium: chloride rats (ie low chloride) prevents excessive secretion of bicarbonate ion. These various ion exchange pumps may result in an alkaline intracellular pH in the presence of selective excessive sodium loading with normal chloride. It was reported that intraerythrocyte sodium content decreased or was in the lower normal pH range and with a significant positive correlation to pH in children on chronic hemodialysis and that there was in addition, an accumulation of sodium and potassium in the erythrocytes during alkalosis. One of the mechanisms resulting in a higher sodium concentration in erythrocytes and muscle in the DOCA-NaAA group, may be metabolic alkalosis. This phenomenon still needs further

investigation. It can be speculated that Kos shows a greater elevation in the DOCA-NaAA group than in the DOCA-NaCl group as a compensation for a high sodium concentration in the tissues. Kos shows a significant inverse correlation with systolic blood pressure in both DOCA treated groups. The elevation in Kos, therefore, may contribute in part to the mechanism for the suppression of the development of hypertension in the DOCA treated rat.

In the DOCA-NaAA group, plasma magnesium concentration was lower and intraerythrocyte magnesium concentration was higher than the Control and the DOCA-NaCl groups. Kos showed a significant positive correlation with intraerythrocyte magnesium concentration and in addition there was a significant inverse correlation between intraerythrocyte magnesium and chloride concentrations in two DOCA treated groups. These relationships among systolic blood pressure, Kos and intraerythrocyte magnesium and chloride concentrations suggest that the activation of the cell membrane Na\(^+\)–K\(^+\) ATPase by high dietary sodium intake in the presence of normal dietary chloride is mediated by intracellular magnesium. The mechanism causing the increase in intracellular magnesium concentration and reduced plasma magnesium concentration in the DOCA-NaAA group compared to the DOCA-NaCl group has not been clarified.

Compared with the DOCA-NaCl group, a reduced norepinephrine turnover rate, despite similar norepinephrine concentration in the heart and the spleen, was noted in the DOCA-NaAA group. The suppressed sympathetic activity may be another factor contributing to the mechanism of suppression of the development of hypertension in the DOCA-NaAA group.

DOCA-salt hypertension depends not only on sodium but also on chloride. Recently, Kurtz T.W. et al.\(^2\) reported that adding sodium bromide and sodium iodide combined to the diet of rats on DOCA could induce an increase in blood pressure. They referred to the DOCA model as "sodium halide-dependant hypertension". The development of hypertension in the DOCA treated rat, therefore, in addition to the provision of sodium, requires the provision of an appropriate anion, such as chloride.

In summary, then, in the DOCA treated rat to which sodium and anions other than chloride have been administered, the blood pressure increases to some extent due to sodium retention and to a lesser extent than in the DOCA-salt rat due to the inhibition of the activation of the sympathetic nervous system and the activation of the membrane sodium pump. The combined effects of sodium and chloride ion are responsible for the DOCA-salt hypertension. The results, to date, present problems for further investigations. The possibility of a hypotensive effect of the NaAA diet containing bicarbonate, phosphate, glutamate and aspartate sodium salts, and the relations of metabolic alkalosis and volume expansion to the difference between the developments of hypertension in the DOCA-NaAA and the DOCA-NaCl groups still need further investigation.

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