ENHANCED RENAL SYMPATHETIC NERVE ACTIVITY IN RESPONSE TO SALT LOADING IN RATS

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To analyze the conflicting data on the relationship between sodium intake and sympathetic activity, the effects of a chronically excessive intake of sodium on renal sympathetic activity and blood pressure were investigated in normotensive rats. Renal sympathetic activity was estimated by urinary excretion of free norepinephrine (NE) and the turnover of NE in the kidneys. Blood pressure increased in rats receiving a high sodium diet when compared with that of the basal sodium diet. Urinary-free NE, epinephrine (E) and dopamine (DA) excretions in rats receiving a high sodium diet were enhanced significantly from those in the basal sodium diet. The turnover of NE in the kidneys was more enhanced in the high sodium group than in the basal sodium group. By blocking the sympathetic tone with ganglionic blockade, hexamethonium, enhanced excretion of urinary NE and elevation of blood pressure in response to salt loading were blocked to the levels of the basal sodium diet. These results suggest that a chronically excessive intake of sodium enhances the renal sympathetic and adreno-medullary activities, leading to a rise in blood pressure in normotensive rats.

CLINICAL and experimental studies have shown that excess dietary sodium is related to an elevation of blood pressure. Increases of blood pressure by an excessive intake of salt were observed in normotensive animals and human subjects as well as in hypertensive animals and patients with essential hypertension. In these studies of hypertensive animals it is speculated that an increase in blood pressure with a high sodium intake is, in part, due to an increase in sympathetic activity; however, it is not clear in normotensive animals and human subjects or hypertensive patients. The major cause of uncertainty, concerning the effect of excess salt intake on sympathetic activity of normotensive animals and human subjects without hypertension, may result in conflicting data of catecholamine release in response to salt loading. Some studies have demonstrated an elevation of norepinephrine (NE) in plasma or urine on sodium loading in normotensive humans or animals. Other studies have revealed no change or a decrease of NE in plasma or urine on sodium loading in normotensive humans or animals. From the above observations of catecholamine analysis, the mechanisms of the hypertensive effect on excess dietary sodium remain unclear.

Key Words:
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Renal Sympathetic Activity and Salt

The present study was conducted to examine the effect of a chronic excessive intake of salt on renal sympathetic nerve activity and to investigate the hypothesis that a chronic excessive intake of salt enhances renal sympathetic activity which, in part, increases blood pressure in normotensive animals.

MATERIALS AND METHODS

Four-weeks old male Wistar rats were purchased from Japan Animal Company and housed in a room kept at a constant temperature (24 ± 2°C). Animals were divided into two groups, and were placed on a diet containing either basal (0.26% sodium, 0.75% potassium) or a high sodium content (3.14% sodium, 0.75% potassium) for 4 weeks. Water and feed were provided ad libitum.

Blood pressure and heart rate were measured using the tail-cuff method and a programmed electro-sphygmomanometer (Narco). Four weeks after the initiation of study, the rats were housed in individual metabolic cages, and after a 24-hour period of acclimatization, 24-hour urine collections were made in flasks containing 1 ml of 6N-HCl to determine the sodium, potassium and catecholamine content. Sodium and potassium contents were determined by flame photometry (Corning, type 480), and results were expressed as mEq/day.

Urinary-free NE, epinephrine (E) and dopamine (DA) contents were determined using high performance liquid chromatography (HPLC) with electrochemical detection (Bioanalytical System Inc.) by a modification of the method of Frayn and Maycock by using the Bond Elut 100-mg SCX cation-exchange columns (Analytichem International) for extraction of catecholamine from the urines. Results were expressed as µg/day (24 hours).

In some of the rats in these two groups, ganglionic blockade with hexamethonium bromide (C6, 40 mg/kg, Nakarai Chemical Co.) was injected intraperitoneously. Blood pressure was measured 1 hour after injection, and then 24-hour urine was collected for NE analysis.

After completion of these studies, NE turnover of the kidneys was estimated by determination of NE content in the kidney before intraperitoneal injection of the tyrosine hydroxylase inhibitor, α-methyl-p-tyrosine (300 mg/Kg, Sigma Chemical Co.), and again 4 hours later and was expressed as percent of mean initial values of renal NE content. After the rats were sacrificed by decapitation without anesthesia, blood was collected and the kidneys were rapidly removed and then frozen on dry-ice and stored at −70°C for a later determination of catecholamine.

Renal catecholamine content was determined using HPLC with electrochemical detection. The kidneys were homogenized with 3 ml/g of cold 0.05M perchloric acid solution containing 0.1 ml of 0.1M EDTA, 0.1 ml of 1M NaHSO4, 45 µl of 0.14 mM DHBA as internal standard. The catecholamine was extracted by a modification of the method of Felice et al.

The blood of the rats, without administration of α-methyl-p-tyrosine, was collected in iced tubes containing EDTA for later determination of plasma renin activity (PRA) using radioimmunoassay (RIA) kits of Clinical Assays and of plasma aldosterone concentration (PAC) using Dainabot RIA kits.

Statistical analysis of the data was performed using Student’s paired and unpaired t comparisons test and expressed in mean ± SEM.

RESULTS

Table I summarized the body weight, blood pressure, heart rate, urinary sodium and potassium excretions, PRA and PAC from the two groups with different sodium intake. There was no significant change in body weight between the two groups of rats. High sodium intake resulted in a marked increase of blood pressure when compared with the rats receiving the basal sodium diet. Heart rate with the high sodium diet was not significantly different from that of the basal sodium diet.

Urinary excretions of sodium and potassium were significantly greater (p < 0.01) in the high sodium rats than in the basal sodium rats. PRA and PAC of rats fed on the high sodium diet were significantly lower than those of the basal sodium diet.

Urinary NE excretion of rats with the high sodium diet (2.17 ± 0.28 µg/day) was significantly higher (p < 0.01) than that of the basal sodium diet (0.71 ± 0.11 µg/day). Urinary E excretion of rats fed on the high sodium diet (0.36 ± 0.03 µg/day) was significantly higher (p < 0.01) than that of the basal sodium diet (0.13 ± 0.02 µg/day). Urinary DA excretion of the high sodium group (3.01 ± 0.49 µg/day) was significantly higher (p < 0.05) than that of the

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TABLE I  BODY WEIGHT, BLOOD PRESSURE, HEART RATE, URINARY SODIUM, URINARY POTASSIUM, PRA (PLASMA RENIN ACTIVITY) AND PAC (PLASMA ALDOSTERONE CONCENTRATION) OF RATS FED ON A BASAL OR HIGH SODIUM DIET

<table>
<thead>
<tr>
<th></th>
<th>Basal sodium</th>
<th>High sodium</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight (g)</td>
<td>223.4 ± 1.3</td>
<td>224.0 ± 2.3</td>
<td>ns</td>
</tr>
<tr>
<td>Blood pressure (mmHg)</td>
<td>113 ± 3.9</td>
<td>135 ± 4.0</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>456 ± 13.1</td>
<td>468 ± 10.7</td>
<td>ns</td>
</tr>
<tr>
<td>Urinary sodium (mEq/day)</td>
<td>0.71 ± 0.14</td>
<td>6.54 ± 1.10</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>Urinary potassium (mEq/day)</td>
<td>1.70 ± 0.16</td>
<td>3.85 ± 0.09</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>3.8 ± 0.9</td>
<td>0.3 ± 0.1</td>
<td>&lt; 0.05</td>
</tr>
<tr>
<td>PAC (pg/ml)</td>
<td>17.4 ± 3.4</td>
<td>2.4 ± 0.4</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

Each value is the mean ± SEM. (n = 10).

Fig. 1. Urinary excretion of norepinephrine (U-NE), epinephrine (U-E) and dopamine (U-DA) measured at 4 weeks after initiation of diets containing either the basal or high sodium diet (n = 10).

basal sodium group (1.40 ± 0.34 μg/day) (Fig. 1). NE content in the kidney was not significantly different between that of the basal sodium rats (76.9 ± 4.5 ng/g) and that of the high sodium rats (73.5 ± 5.3 ng/g). DA content of the kidneys receiving a high sodium diet (22.6 ± 0.7 ng/g) was significantly higher (p < 0.01) than the basal sodium diet (15.5 ± 2.8 ng/g) (Fig. 2).

NE turnover in the kidneys, expressed as percent of mean initial values of renal NE content after the blockade of synthesis with α-methyl-p-tyrosine, was significantly enhanced (p < 0.01) in rats fed on the high sodium diet (35.0 ± 4.2%) more than that of the basal sodium diet (58.9 ± 6.4%) (Fig. 3).

Under blocking the peripheral sympathetic activity with hexamethonium, blood pressure was decreased significantly in both groups of rats. Elevated blood pressure in response to high sodium diet disappeared after ganglionic blocking. Urinary excretion of NE was decreased significantly in both groups of rats. Enhanced excretion of urinary NE in response to the high sodium diet also disappeared after ganglionic blocking (Table II).

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DISCUSSION

The present study demonstrated that chronic excessive intake of sodium elicited an overactivity of renal sympathetic nerves in normotensive rats. These results are compatible with the direct measurement of the sympathetic neural firing in rats fed chronically on a high sodium diet, in which Sasaki and Bunag\textsuperscript{16} clearly demonstrated the overactivity of the sympathetic nerve in chronic salt loading. In the present study, we measured the NE turnover in the kidneys, which is a better index of NE neural activity\textsuperscript{14} when compared with plasma level or urinary excretion of NE, and showed that NE turnover in the kidneys of rats with the high sodium diet was definitely enhanced more than that of rats with the basal sodium diet. In experimental hypertensive rats, it is reported that NE turnover increases in the heart, while it decreases in the brain.

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Therefore the excessive intake of sodium in normotensive animals elicits the increase of sympathetic nerve activity including renal sympathetic activity, and augments the excretory amount of NE into urine, which is almost identical with the enhancement of sympathetic nerve activity in experimental hypertensive rats. Moreover, the urinary-free NE excretion may mainly represent NE released from the renal sympathetic nerve in the kidneys, although circulating free NE is, in part, excreted into the urine.

The renal sympathetic nervous system has an important role in the regulation of sodium and water balance, and the enhanced renal sympathetic activity increased the renal tubular sodium reabsorption and produced significant sodium retention in the body. Therefore, the augmented sodium retention of rats with a high sodium diet was elicited by the overactivity of the sympathetic nerve, although the renal perfusion pressure might be increased. Additionally, the high dietary sodium intake was associated with an increase of urinary excretion of potassium. Previous study indicated that dietary sodium excess leads to a negative potassium balance and a decreased total body potassium. Therefore it is suggested that pressor effect and enhanced sympathetic nerve activity by excess intake of salt are, in part, due to potassium depletion to sodium loading.

Enhanced sympathetic activity in response to high sodium diet was suppressed by ganglionic blockade with hexamethonium. The elevation of blood pressure and augmentation of urinary NE excretion of the high sodium group disappeared by hexamethonium. These results suggested that the elevation of blood pressure was enhanced by the augmentation of sympathetic activity.

Although the activating site and mechanism in sympathetic activity are not clear, our laboratory has already demonstrated that sympathetic overactivity and enhanced pressor responses were elicited by an increased concentration of NaCl in cerebro-spinal fluid with an intracisternal injection of hypertonic NaCl. From these observations, it is clear that the excessive intake of sodium can elicit sympathetic overactivity in the central nervous system.

As previously reported, however, there are conflicting data on the relationship between excessive intake of sodium and NE level in plasma or urine. Some studies have demonstrated an elevation of NE in plasma or urine on sodium loading in normotensive humans or animals. Other studies have revealed no change or a decrease of NE in plasma or urine on sodium loading in normotensive humans or animals. Similar inconsistencies appear in the relationship between sodium intake and E or DA. Both an increase and a decrease of E level in plasma or urine on salt loading, as well as an increase and a decrease of DA level in plasma or urine on salt loading have been recorded.

In analyzing these conflicting data, the amount of sodium loading, duration of sodium loading and susceptibility of animals and humans to sodium may be responsible for these different results.

To further explore and clarify the effect of excess dietary sodium on sympathetic activity and catecholamine release, we investigated the chronic effect of the excessive intake of sodium on sympathetic neural firing and catecholamine release into urine and found that sympathetic activity and urinary NE excretion were influenced by the baroreceptor reflex. In the early stage (within 2 weeks) of salt loading, the sympathetic neural firing and NE release into urine were suppressed by an enhanced cardio-pulmonary baroreceptor reflex, which was caused by an increase in the circulating blood volume in the developing stage of a salt induced elevation in blood pressure. However in the chronic stage of salt loading, the sympathetic neural firing and NE release into urine were enhanced greatly when compared to those rats with the basal sodium diet.

These results suggest that the enhanced sympathetic activity elicited by an excessive intake of salt could be suppressed by the cardio-pulmonary baroreceptor reflex which may be elicited by afferent input from an increase in the circulating blood volume by salt loading. In addition, in the chronic stage of salt loading, enhanced sympathetic activity may be greater than the intensity of the cardio-pulmonary baroreceptor reflex, thus, urinary excretion of NE increases in this stage. These fluctuations in sympathetic activity, which are dependent on the duration of salt loading, could possibly cause the conflicting data, concerning the relationship between the excessive intake of salt and plasma level or urinary excretion of NE. Similar observations were reported that NE released into blood in response to saline infusion was enhanced more by bilateral vagotomy when compared with that
of intact animals.

Another factor to consider in viewing conflicting data is the susceptibility to sodium in the NE level in plasma or urine. Available evidence suggested that subgroups of patients with essential hypertension were identified as salt-sensitive or nonsalt-sensitive patients according to their pressor response to salt loading. In salt-sensitive patients, the plasma NE level was significantly higher in salt loading when compared with that of the nonsalt-sensitive patients. The susceptibility to sodium in NE release may relate to the inconsistencies in the relationship between sodium intake and NE level in plasma or urine.

Increased excretion of urinary E showed a pattern similar to like those of NE, which indicated that E excretion from the adrenal medulla in response to sodium intake is mediated via the sympathetic nervous system. In addition, E stimulates the release of NE from the sympathetic nerve by activating prejunctional β-adrenoceptors. Therefore increased sympatho-adrenal activity contributes to elevate blood pressure.

Urinary excretion of DA in response to salt loading is believed to be facilitated when accompanied with an increase of sodium excretion. In the present study, urinary excretion of DA was enhanced with the excessive intake of sodium and the release of free DA in response to salt loading may be due to the overactivity of the renal sympathetic nerve, as well as an elevated DA formation in the renal tubulus.

Although urinary excretions of free NE and free DA were increased in response to salt loading, renal content of NE and DA responded to salt loading in different ways, such as no change of renal NE content and an increase of renal DA content. These results suggest that increased synthesis and release of NE are independent from those of DA and that DA is not only the precursor of NE but also a transmitter that responds to salt loading to release independently from the response of NE.

From these results it is concluded that a chronically excessive intake of sodium enhances the renal sympathetic neural activity estimated by increased urinary excretion of free NE and the enhanced turnover of NE in the kidneys. The overactivity of the renal sympathetic nervous system and of the adrenal medulla contribute to elevate blood pressure in chronic salt loading.

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