Role of Cellular Calcium in Salt Sensitivity of Patients with Essential Hypertension

TETSUYA OSHIMA, Hideo Matsuura, Koji Matsumoto, Koji Kido, AND GORO KAJIYAMA

SUMMARY The mechanism by which excessive sodium chloride intake raises blood pressure has not been fully clarified. The present study was therefore undertaken in patients with essential hypertension to investigate the possible role of an intracellular calcium-dependent mechanism in salt sensitivity. The difference in mean blood pressure between a week of low sodium chloride diet (3 g/day) and a week of high sodium chloride diet (20 g/day) was studied in relation to the intracellular free calcium concentration in lymphocytes and an acute hypotensive response to a 10-mg sublingual dose of nifedipine in 12 inpatients. Sodium chloride loading induced significant increases in mean blood pressure (from 111 ± 12 to 122 ± 11 mm Hg; p<0.01), intracellular free calcium in lymphocytes (from 133 ± 13 to 145 ± 9 nmol/L; p<0.01), and the hypotensive response to nifedipine (from 19 ± 6 to 31 ± 10 mm Hg; p<0.01). In addition, serum total calcium concentration was decreased while urinary calcium excretion was increased. The elevation of mean blood pressure was closely and positively correlated with the increase in intracellular free calcium concentration (r=0.71, p<0.05) and the increase in the hypotensive effect of nifedipine (r=0.91, p<0.01) after sodium chloride loading. However, changes in these values had no relation to the change in serum concentration or urinary excretion of calcium. These data suggest that change in the cellular calcium-dependent vasoconstriction mechanism may be associated with salt sensitivity of patients with essential hypertension.

KEY WORDS salt sensitivity • essential hypertension • intracellular free calcium concentration • lymphocytes • nifedipine

SODIUM chloride is well known to be an important factor in the pathogenesis and development of essential hypertension. However, excessive sodium chloride intake does not always raise blood pressure, as several reports have shown marked interindividual differences in response of blood pressure to changes in dietary salt intake (i.e., salt sensitivity) in patients with essential hypertension. The actual mechanism underlying the differences in salt sensitivity has not been fully clarified, although expansion of fluid volume, inappropriate response of the hormonal factors, and enhanced vascular response to pressor substances have been proposed to explain the mechanism.

Since the importance of intracellular free calcium concentration ([Ca\(^{2+}\)]) in cellular functions of many cells involved in blood pressure regulation, such as vascular smooth muscle cells, has been recognized, interest has been focused on the role of alterations in the cellular calcium metabolism in the pathogenesis of essential hypertension. Many investigators have reported disturbances in the calcium transport system across the cell membrane and intracellular binding process and elevation of [Ca^2+] in circulating blood cells of patients with essential hypertension. In addition, clinical findings of enhanced hypotensive response to calcium channel blockers in this disease provide suggestive evidence for an abnormality in cellular calcium movements.

However, there is limited information regarding the effects of dietary salt intake on cellular calcium metabolism and [Ca\(^{2+}\)], and their relation to salt sensitivity. Therefore, to assess the possible role of calcium influx or [Ca^2+]-dependent mechanism in salt sensitivity of patients with essential hypertension, the [Ca^2+] in lymphocytes and the acute blood pressure response to sublingual doses of nifedipine, which inhibits calcium
influx with relative selectivity for vascular smooth muscle, were determined in 12 inpatients with essential hypertension during intake of low sodium chloride diet and intake of high sodium chloride diet.

**Patients and Methods**

Twelve Japanese inpatients with mild to moderate essential hypertension (7 men and 5 women) with a mean age of 54.5 years (range, 42–63 years) were studied. All the patients gave informed consent to this study. The presence of hypertension was defined as a blood pressure level of greater than 160/95 mm Hg in the sitting position on at least three different occasions in the outpatient clinic. Patients with secondary forms of hypertension were ruled out by appropriate clinical and laboratory examinations. No medication was permitted for at least 4 weeks before the study.

All the patients were on a constant daily diet of 10 g of sodium chloride for 7 days to stabilize the blood pressure and sodium balance before the study began. This phase was followed by 7 days of a low sodium chloride diet (3 g/day) and then by 7 days of a high sodium chloride diet (20 g/day). The high sodium chloride diet was achieved by adding Slow Sodium tablets (600 mg of sodium chloride per tablet; CIBA, Horsham, UK) to the diet. Throughout the study, the patients ingested a constant amount of potassium (2000 mg/day), calcium (400 mg/day), and calories (40 Cal/kg). A 24-hour urine collection was performed every day for determination of sodium, potassium, and calcium excretions. On the seventh morning of each diet, the fasting patient maintained a supine position in a quiet and dark room and an indwelling cannula was inserted into an antecubital vein. Thirty minutes later, blood pressure and heart rate were measured and a venous sample was obtained for the determination of serum sodium, potassium, and calcium concentrations, plasma norepinephrine concentration, plasma renin activity, and 

\[ \text{[Ca}^{2+}] \text{]i in lymphocytes. Blood pressure measurements were performed with a sphygmomanometer every minute for 10 minutes, and the average of 10 consecutive blood pressure readings was taken as the basal value of each diet period. Mean blood pressure was calculated as the sum of diastolic blood pressure and one third of pulse pressure. After the quin 2 loading procedure, the lymphocytes were resuspended in fresh RPMI-1640 and left at room temperature for 60 minutes. The cells were centrifuged at 400 g for 10 minutes and resuspended (3–5×10^6 cells/ml) in a saline solution consisting of (mM) NaCl, 145; KCl, 5; Na HEPES, 10; NaHPO_4, 1; CaCl_2, 1; MgSO_4, 0.5; glucose, 5; pH 7.40 at 37°C. Fluorescence was determined at a 339-nm excitation wavelength (bandwidth, 4 nm) and a 492-nm emission wavelength (bandwidth, 10 nm) using a spectrofluorometer (Hitachi 204S, Tokyo, Japan) at 37°C. 

\[ \text{[Ca}^{2+}] \text{]i in lymphocytes was determined with quin 2 according to the method described by Tsien et al. with slight modifications. The lymphocytes were suspended (1–3×10^7 cells/ml) in RPMI-1640 containing 50 μM quin 2 tetraacetoxymethyl ester (quin 2 AM, Dojindo Laboratories, Kumamoto, Japan) and then incubated at 37°C for 30 minutes. After the quin 2 loading procedure, the lymphocytes were resuspended in fresh RPMI-1640 and left at room temperature for 60 minutes. The cells were centrifuged at 400 g for 10 minutes and resuspended (3–5×10^6 cells/ml) in a saline solution consisting of (mM) NaCl, 145; KCl, 5; Na HEPES, 10; NaHPO_4, 1; CaCl_2, 1; MgSO_4, 0.5; glucose, 5; pH 7.40 at 37°C. Fluorescence was determined at a 339-nm excitation wavelength (bandwidth, 4 nm) and a 492-nm emission wavelength (bandwidth, 10 nm) using a spectrofluorometer (Hitachi 204S, Tokyo, Japan) at 37°C. 

Results

Mean blood pressure, heart rate, [Ca^{2+}]i in lymphocytes, serum concentrations of sodium, potassium, and calcium, urinary excretions of sodium, potassium, and calcium (mean of the last 2 days in each period), plasma renin activity, plasma norepinephrine concentration, and effects of nifedipine during low and high sodium chloride periods are shown in Table 1. Mean blood pressure and [Ca^{2+}]i in lymphocytes were signficantly increased with high sodium chloride diet. Both plasma renin activity and plasma norepinephrine concentration significantly increased with high sodium chloride diet.
TABLE 1. Clinical and Laboratory Data and Effects of Nifedipine Treatment During Low and High Sodium Chloride Intakes

<table>
<thead>
<tr>
<th>Variable</th>
<th>Low NaCl</th>
<th>High NaCl</th>
</tr>
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<tbody>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>111.2 ± 12.4</td>
<td>121.7 ± 11.4*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>62.3 ± 10.7</td>
<td>59.6 ± 7.1</td>
</tr>
<tr>
<td>Lymphocyte [Ca^{2+}] (nmol/L)</td>
<td>132.9 ± 12.8</td>
<td>145.4 ± 9.3*</td>
</tr>
<tr>
<td>Serum sodium concentration (mmol/L)</td>
<td>144.3 ± 3.3</td>
<td>143.5 ± 2.6</td>
</tr>
<tr>
<td>Serum potassium concentration (mmol/L)</td>
<td>4.23 ± 0.23</td>
<td>4.18 ± 0.30</td>
</tr>
<tr>
<td>Serum calcium concentration (mmol/L)</td>
<td>2.32 ± 0.09</td>
<td>2.24 ± 0.08†</td>
</tr>
<tr>
<td>Plasma renin activity (ng Ang I/ml/hr)</td>
<td>2.23 ± 2.20</td>
<td>0.45 ± 0.38*</td>
</tr>
<tr>
<td>Plasma norepinephrine level (pg/ml)</td>
<td>152 ± 77</td>
<td>102 ± 59†</td>
</tr>
<tr>
<td>Urinary sodium excretion (mmol/day)</td>
<td>52.5 ± 10.9</td>
<td>334.3 ± 19.4*</td>
</tr>
<tr>
<td>Urinary potassium excretion (mmol/day)</td>
<td>44.4 ± 9.9</td>
<td>45.8 ± 9.6</td>
</tr>
<tr>
<td>Urinary calcium excretion (mmol/day)</td>
<td>2.60 ± 1.88</td>
<td>4.29 ± 2.18*</td>
</tr>
<tr>
<td>Responses to nifedipine</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>−19.1 ± 6.4</td>
<td>−31.4 ± 9.6*</td>
</tr>
<tr>
<td>Heart rate (beats/min)</td>
<td>10.8 ± 3.7</td>
<td>16.4 ± 6.2†</td>
</tr>
<tr>
<td>Plasma renin activity (ng Ang I/ml/hr)</td>
<td>0.40 ± 0.36</td>
<td>0.72 ± 0.38†</td>
</tr>
<tr>
<td>Plasma norepinephrine level (pg/ml)</td>
<td>81 ± 48</td>
<td>120 ± 79*</td>
</tr>
</tbody>
</table>

Values are means ± SD. [Ca^{2+}] = intracellular Ca^{2+} concentration; Ang I = angiotensin I.

*p < 0.01, †p < 0.05, compared with values during low sodium chloride period.

concentration were suppressed after sodium chloride loading. Heart rate and serum concentrations of sodium and potassium and urinary potassium excretion did not differ between low and high sodium chloride periods. Urinary excretion of sodium showed good adherence to the regimens. Urinary calcium excretion was increased significantly, and serum total calcium concentration showed a small but significant decrease. Sodium chloride loading augmented the hypotensive response and increases in heart rate, plasma renin activity, and plasma norepinephrine concentration with nifedipine treatment. The maximum fall in mean blood pressure was observed within 10 to 25 minutes after administration of nifedipine in all the patients, and this duration was not changed after sodium chloride loading. In addition, the lowest value of mean blood pressure after nifedipine was similar in both diets.

During both low and high sodium chloride periods, basal mean blood pressure was not correlated with the hypotensive response to nifedipine (r = 0.04, not significant [NS] in low sodium chloride diet; r = 0.38, NS in high sodium chloride diet) or [Ca^{2+}], in lymphocytes (r = 0.19, NS in low sodium chloride diet; r = 0.05, NS in high sodium chloride diet). There was a positive relation between [Ca^{2+}], in lymphocytes and the fall in mean blood pressure with nifedipine treatment during both low (r = 0.62, p < 0.05) and high (r = 0.66, p < 0.05) sodium chloride diets. Salt sensitivity, regarded as the difference in basal mean blood pressure between low and high sodium chloride diets, showed a close and positive correlation with change in [Ca^{2+}], in lymphocytes (r = 0.71, p < 0.05; Figure 1A) and with change in the hypotensive response to nifedipine treatment (r = 0.91, p < 0.01; Figure 1B) after sodium chloride loading. Changes in basal mean blood pressure, the hypotensive response to nifedipine treatment, and [Ca^{2+}], in lymphocytes showed no relation to change in serum concentration or urinary excretion of calcium, plasma renin activity, or plasma norepinephrine concentration after sodium chloride loading.

Discussion

In the present study, the hypotensive response to nifedipine was found to be a useful tool for the assessment of in vivo cellular calcium metabolism in vascular smooth muscle. Nifedipine has been considered to induce a potent vasodilation resulting mainly from inhibition of transmembranous calcium influx into vascular smooth muscle. Therefore, the acute hypotensive response to nifedipine may reflect in part cellular calcium influx–dependent vasoconstriction. The present study demonstrated that sodium chloride loading enhances the hypotensive property of this drug. However, the factors determining the magnitude of the

![Figure 1](http://hyper.ahajournals.org/Downloadedfrom.png)

**Figure 1.** Relation of change in basal mean blood pressure (salt sensitivity) to change in intracellular Ca^{2+} concentration ([Ca^{2+}]_{i}) in lymphocytes (A) and to change in the hypotensive response to nifedipine treatment (B) after sodium chloride loading.
acute blood pressure response to nifedipine are complex. Experimental and clinical findings have shown that nifedipine partially attenuates norepinephrine-induced and angiotensin II-induced vasoconstriction. However, enhancement of the hypertensive effect of nifedipine after sodium chloride loading probably was not dependent on vasoconstriction induced by these hormones, since high sodium chloride diet suppressed plasma renin activity and plasma norepinephrine concentration. In addition, the acute hypertensive response to nifedipine is accompanied by significant increases in heart rate, plasma renin activity, and plasma norepinephrine concentration as a consequence of baroreceptor reflex-mediated sympathetic activation. Changes in these counterregulatory mechanisms may influence the antihypertensive effect of nifedipine. However, increases in heart rate, plasma renin activity, and plasma norepinephrine with nifedipine administration were significantly greater in the high sodium chloride period than in the low sodium chloride period. The possibility that sodium chloride loading disturbed the activation of these counterregulatory mechanisms in response to the fall in blood pressure seems unlikely. It has been considered that the fall in blood pressure after the administration of any agent depends directly on the pretreatment level. It is impossible to deny that after sodium chloride loading the enhanced hypertensive response to nifedipine resulted in part from an elevation of pretreatment blood pressure. However, the effects of most antihypertensive agents other than calcium channel blockers and diuretics are potentiated by a low sodium chloride diet and blunted by a high sodium chloride diet. Furthermore, nifedipine lowered mean blood pressure to a similar level in both the low and the high sodium chloride periods and salt sensitivity was closely and positively correlated with the change in the nifedipine-induced reduction of blood pressure after the sodium chloride loading. Thus, salt sensitivity may be associated with changes in vasoconstriction mechanisms that are susceptible to nifedipine.

One other important difference between low and high sodium chloride diets was shown in [Ca\(^{2+}\)], in lymphocytes. In addition, an increase in lymphocyte [Ca\(^{2+}\)], was positively linked with elevation of mean blood pressure after sodium chloride loading. If lymphocytes reflect a similar alteration of [Ca\(^{2+}\)], in vascular smooth muscle cells, a considerable proportion of the pressor effect of sodium chloride loading may be due to an elevation of [Ca\(^{2+}\)]. However, the precise mechanism whereby a high sodium chloride diet raises [Ca\(^{2+}\)], in lymphocytes is unclear. Two hypotheses have been advanced to account for the increase in [Ca\(^{2+}\)], after sodium chloride loading. One is the sodium transport hypothesis proposed by de Wardener and MacGregor. Blaustein, and many others. According to this hypothesis, a circulating Na\(^{+},K\(^{+}-\)adenosine triphosphatase inhibitor stimulated by volume expansion may increase intracellular sodium concentration and thereby raise [Ca\(^{2+}\)], through an alteration of the Na\(^{+}-Ca\(^{2+}\) exchange process. However, many investigators have failed to demonstrate the physiologic importance of the Na\(^{+}-Ca\(^{2+}\) exchange process in regulating [Ca\(^{2+}\)], in lymphocytes by using quin 2, while a \(^{45}\)Ca flux study has shown the existence of the Na\(^{+}-Ca\(^{2+}\) exchange system in rat thymocytes and in rabbit lymphocyte plasma membranes.

Alternatively, Resnick et al. proposed that alterations in calcium-regulating hormones (parathyroid hormone, calcitonin, and 1,25-dihydroxyvitamin D) after sodium chloride loading may lead to the distribution of calcium from outside the cell to inside the cell. In fact, lymphocytes have been reported to possess the receptor of parathyroid hormone and calcitonin. In addition, our finding that urinary calcium excretion was increased and serum total calcium concentration was decreased with a high sodium chloride diet implies a negative effect of sodium loading on systemic calcium balance. Kurtz et al. demonstrated in a small number of patients with essential hypertension that sodium chloride supplementation increased urinary calcium excretion and blood pressure, while sodium citrate did not. The pressor response to sodium chloride may be due in part to an adverse effect of chloride on calcium homeostasis. The negative calcium balance could modify the calcium-regulating hormones. However, the changes in serum and urinary calcium after sodium chloride loading did not show any relation to the changes in basal mean blood pressure, the hypertensive response to nifedipine, or the [Ca\(^{2+}\)], in lymphocytes in the present study. Therefore, we could not clarify the direct link of the alteration in systemic calcium balance with intracellular calcium accumulation or salt sensitivity.

In conclusion, mean blood pressure, the hypertensive response to nifedipine, and [Ca\(^{2+}\)], in lymphocytes were increased with changes in sodium chloride intake from 3 to 20 g/day in patients with essential hypertension. The change in mean blood pressure was positively correlated with changes in the hypertensive response to nifedipine and [Ca\(^{2+}\)], in lymphocytes after sodium chloride loading. These results suggest that enhancement of a cellular calcium-dependent vasoconstriction mechanism may result in elevation of blood pressure in response to sodium chloride loading in essential hypertension.

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