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Dietary NaCl Loading Increases Platelet Ca^{2+} Discharge Capacity in Salt Resistant Essential Hypertension

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ABSTRACT

To clarify the relationship between cellular Ca^{2+} handling and salt sensitivity, we evaluated cytosolic free Ca^{2+} ($[Ca^{2+}]i$) in fura-2 loaded platelets isolated from 20 inpatients with essential hypertension. They were placed on a low sodium diet (50 mmol/day) for one week, followed by one week on a high sodium diet (340 mmol/day). They were classified into salt-sensitive (SS, n=8) or salt-resistant (SR, n=12) based on changes in the mean blood pressure. During the low salt diet, basal $[Ca^{2+}]i$, thrombin-evoked maximal Ca^{2+} responses, irrespective of the presence of 1 mM extracellular Ca^{2+}, and ionomycin-sensitive intracellular Ca^{2+} discharge capacity were similar in salt-sensitive and salt-resistant patients. Platelet basal $[Ca^{2+}]i$ were increased in both groups by salt loading (SS, from 22.0 ± 1.3 to 27.2 ± 1.9 nM, $p<0.01$; SR, from 20.1 ± 0.8 to 24.4 ± 1.3 nM, $p<0.05$). The thrombin-evoked maximal Ca^{2+} responses both in the presence and absence of extracellular Ca^{2+} were unchanged by high salt intake. The rate constant of decline in Ca^{2+} after the peak response to thrombin was larger in SR than that in SS during the high salt diet period (SS, 0.004 ± 0.001 sec^{-1}; SR, 0.043 ± 0.014 sec^{-1}, $p<0.05$). The intracellular Ca^{2+} discharge capacity was increased by excessive salt intake in the salt-resistant patients but was unchanged in the salt-sensitive patients (SS, from 658.1 ± 52.8 to 639.6 ± 91.9 nM; SR, from 690.8 ± 65.1 to 803.3 ± 65.1 nM, $p<0.05$). An increased intracellular Ca^{2+} discharge capacity may play, at least in part, a significant role in preventing the elevation of blood pressure after salt loading in salt-resistant patients with essential hypertension.

***Key words**: Salt sensitivity, Calcium, Platelet, Essential hypertension*

Epidemiologic studies indicate that salt intake is an important environmental factor in the pathogenesis and development of essential hypertension[7,14,17]. However, excessive salt intake does not increase the blood pressure of all patients with essential hypertension[16]. The mechanism for the difference in salt sensitivity is not fully understood. Inappropriate adaptation of the renin-angiotensin system[32], sympathetic nerve activity[4], endogenous digitalis-like factor(s)[10], and/or calcium-regulating hormones[3,13] may all be involved in the pressor response to salt loading in essential hypertension. Recent studies have shown that changes in cellular ionic handling are related to the mechanism of salt sensitivity[1,5,10,25,28,34]. Salt loading has been reported to increase the intracellular Na^+ concentration and reduce the intracellular pH in salt-sensitive essential hypertension[28]. An excessive intake of salt also increases the intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) in lymphocytes and platelets[1,5,25,28,34]. These studies mentioned the effects of a high salt intake on $[Ca^{2+}]i$ only in a resting state. However, the role of Ca^{2+} handling in the pathophysiology of hypertension should be evaluated in actively functioning cells, not in resting cells.

This study was designed to elucidate the effect of dietary salt intake on platelet $[Ca^{2+}]i$ handling in patients with essential hypertension. We evaluated the Ca^{2+} handling in response to thrombin both in the presence and absence of extracellular Ca^{2+} as well as the ionomycin-sensitive intracellular Ca^{2+} discharge capacity which is considered to reflect intracellular Ca^{2+} storage size.

MATERIALS AND METHODS

Subjects

Twenty Japanese patients with mild to moderate essential hypertension (13 men and 7 women, mean age 52.1 ± 12.6 years) were enrolled in the study. Hypertension was defined as a systolic blood pressure level of greater than 160 mmHg and/or a diastolic level of greater than 95 mmHg that was obtained on at least three different occasions with the patients seated in the outpatient clinic. Patients with secondary forms of hypertension were excluded from the study by appropriate clinical and laboratory examinations. None of the patients had ischemic heart disease, cerebrovas-

cular stroke or other vascular complications. All patients provided their informed consent to participate in the following protocol.

Protocol

Patients were admitted to the First Department of Internal Medicine, Hiroshima University after interruption of antihypertensive medications for at least 4 weeks. All patients were placed on a regular salt diet containing 170 mmol/day of sodium for 7 to 10 days to stabilize the blood pressure and the sodium balance. After the period on the regular salt diet, the patients were given a low salt diet (50 mmol/day of sodium) for 7 days, followed by a high salt diet (340 mmol/day) for 7 days. The high salt diet was prepared by supplementing the regular salt diet with sodium chloride tablets (10 mmol/tablet; Slow-Salt, San Antonio, TX, USA). The intake of other dietary elements and calories was kept constant (100 mmol/day of potassium, 40 mmol/day of calcium, and 40 Cal/kg). A 24-hour urine collection was performed daily to monitor the excretion of sodium and potassium. Blood pressure was measured twice a day throughout the study. None of the patients revealed severe elevation of blood pressure accompanied by serious headache. On the last morning of each diet period, the patients fasted overnight, and rested supine in a dark, quiet, and air-conditioned room for at least 30 minutes. The blood pressure and heart rate were measured and samples of venous blood were obtained to determine serum levels of sodium, potassium, and calcium concentrations, as well as for the determination of $[Ca^{2+}]i$ in platelets. Blood pressure measurements were performed with a mercury sphygmomanometer. The average of 10 consecutive blood pressure readings was used as the representative value for each diet period. The mean blood pressure was calculated as the sum of diastolic blood pressure and one third of pulse pressure. Patients whose percent change in mean blood pressure exceeded 10% when changing from a low to a high salt diet were defined as salt-sensitive (SS), and the remainder were defined as salt-resistant (SR). Serum and urinary electrolyte concentrations were measured with an autoanalyzer (PVA-αII, Central SC, Osaka, Japan).

Intracellular calcium measurement

To obtain platelet-rich plasma, blood samples were centrifuged at 800 *g* for 5 min at room temperature. Platelet-rich plasma was gel-filtered through a Sepharose 2B-CL column (Pharmacia LKB, Uppsala, Sweden) which had been pre-equilibrated with Ca^{2+}-free buffer containing 145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 10 mM 2-[4-(2-Hydroxyethyl)-1-piperazinyl] ethanesulfonic acid (HEPES), and 5 mM d-glucose, pH 7.4. The washed platelet solution was adjusted to a platelet count of 10^8 cells/ml and incubated with 1 μM of fura-2/acetoxymethylester (AM; Molecular Probes, Eugene, Oregon, USA) and 0.02% Pluronic F-127 (Molecular Probes) for 30 min at 37°C. After additional gel filtration to remove extraneous fura-2/AM, the platelets were diluted with the above buffer to a final concentration of 10^7 cells/ml. $CaCl_2$ was added to this cell suspension to achieve a final concentration of 1 mM.

For the measurement of fluorescence, a 2.5 ml aliquot of the cell suspension was stirred in a quartz cuvette at 37°C. Fluorescence was recorded with a spectrofluorophotometer (DM3000CM, SPEX Industries, Edison, NJ, USA) by using excitation wavelengths of 340 and 380 nm and an emission wavelength of 510 nm. After the basal fluorescence was measured, the $[Ca^{2+}]i$ responses to 0.03～1.0 U/ml of thrombin (Sigma Chemical Co., St. Louis, MO, USA) were observed both in the presence of 1 mM extracellular Ca^{2+} and in a nominally free Ca^{2+} buffer which was prepared by the addition of 10 mM of ethyleneglycol-bis-(β-aminoethylester)-N,N,N',N',-tetraacetic acid (EGTA; Dojindo Laboratories, Kumamoto, Japan) into the buffer containing 1 mM Ca^{2+}, pH 7.4. Analysis of the kinetic of the recovery of Ca^{2+} (Ca^{2+} uptake and extrusion) in thrombin-stimulated platelets in the absence of extracellular Ca^{2+} was performed as previously described[26]. We calculated the Ca^{2+} transient at 10 second intervals for 40 seconds after peak response to 0.3 U/ml of thrombin. By using the five data points taken in 40 seconds for each experiment, the Ca^{2+} transient equation $[Ca^{2+}]i=Ae^{-kt}$ was derived by regression analysis, where A represents compartment size, k the rate constant and t time (second). We also assessed the response of $[Ca^{2+}]i$ to 5 μM of ionomycin (Sigma Chemical Co.) in the absence of extracellular Ca^{2+} as an index of the intracellular Ca^{2+} discharge capacity[26].

Fluorescence was corrected for extracellular dye leakage with EGTA and for autofluorescence by subtracting the fluorescence of the unloaded platelets, buffer, and cuvette[27]. After 10 mM of EGTA was added to the intact cell suspension at pH 7.4, a rapid initial drop in the fluorescence signal at 340 nm was considered to reflect the contribution by the extracellular dye as extracellular calcium was chelated. After lysis of the cells by addition of 50 μM of digitonin to estimate the total fura-2 associated fluorescence, the ratio of fluorescence change after EGTA at pH 7.4 in intact cell suspension to that in the total dye was regarded as the percentage of dye leaked from the cell. The fluorescent signal of the external dye was then subtracted from the original signal. The total fura-2 fluorescence signal was converted to a concentration of fura-2 in cells using standard curve, platelet count, and mean platelet

volume assessed by an automatic counter (STKS, Coulter Japan, Tokyo, Japan).

The cytosolic Ca^{2+} concentration was calculated by a general formula as follows[9]: $[Ca^{2+}]i$ (nM) = 224 nM × (R-Rmin)/(Rmax-R) × β, where 224 nM is the dissociation constant of Ca^{2+}-fura-2 complex and R represents the ratio of 340/380 fluorescence; Rmax is the maximal fluorescence rate in the Ca^{2+}-supplemented (1 mM) and Rmin is minimal fluorescence rate in the Ca^{2+}-depleted condition by addition of 10 mM of EGTA at pH 8.3 adjusted with Tris, and β is the ratio of fluorescence value at 380 nm in the absence of Ca^{2+} to the value in the presence of saturating Ca^{2+}.

Statistics

Data are expressed as mean ± SEM. Comparisons of the parameters on the low vs. high salt diet periods were performed by using Wilcoxon's signed rank test. The Mann-Whitney U test was used to compare the values between SR and SS. A level of $p<0.05$ was accepted as statistical significant.

RESULTS

The patients' characteristics are shown in Table 1. Eight patients were classified as SS and the remaining 12 as SR. There were no significant differences in age, gender distribution, body weight, blood pressure, and mean platelet volume during the low salt diet period between the two groups. Blood pressure increased only in the SS group. Body weight increased both in SR and SS patients during the high salt diet. The mean platelet volume was not changed by salt loading. Serum concentration and urinary excretion of electrolytes on both dietary regimens were similar in SS and SR patients. The serum concentration of potassium decreased and the urinary excretion of sodium and calcium increased due to salt loading in both SS and SR patients. Changes in these parameters did not differ significantly between the two groups. The serum concentration of sodium increased in SR patients after salt loading.

Table 1. Characteristics of Salt-Resistant and Salt-Sensitive Patients Administered Low and High Salt Diets

Parameters	Salt-Resistant (n=12)		Salt-Sensitive (n=8)	
	Low Salt	High Salt	Low Salt	High Salt
Age (years)	51.1 ± 3.8		53.8 ± 3.9	
Gender (male/female)	9 / 3		4 / 4	
Mean BP (mmHg)	103.5 ± 2.8	105.6 ± 2.8	101.5 ± 2.7	120.5 ± 3.6*†
Heart rate (bpm)	61 ± 1	64 ± 3	60 ± 3	58 ± 4
Body weight (Kg)	69.7 ± 2.7	70.6 ± 2.8*	65.1 ± 3.7	65.8 ± 4.5*
Mean platelet volume (fl)	9.6 ± 0.7	9.7 ± 0.6	9.5 ± 0.4	9.5 ± 0.5
Serumconcentration of				
sodium (mM)	140.5 ± 0.7	142.0 ± 0.9*	140.3 ± 0.9	141.0 ± 0.9
potassium (mM)	4.6 ± 0.1	4.1 ± 0.1**	4.7 ± 0.1	4.3 ± 0.2*
calcium (mM)	2.4 ± 0.1	2.3 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Urinary excretion of				
sodium (mmol/day)	51.8 ± 3.8	298.6 ± 16.2**	47.1 ± 5.1	315.7 ± 14.3**
potassium (mmol/day)	71.4 ± 7.4	68.1 ± 4.7	65.1 ± 6.9	68.8 ± 7.2

*p < .05, **p < .01 vs. low salt diet. †p < .05 vs. salt resistant
Data are expressed as mean ± SEM.

Table 2. Metabolism of Fura-2 during Low and High Salt Diet Periods

Parameters	Salt-Resistant		Salt-Sensitive	
	Low Salt	High Salt	Low Salt	High Salt
[fura-2]i (μM)	60.9 ± 7.4	68.1 ± 11.2	64.0 ± 11.7	70.1 ± 5.9
dye leakage (%)	12.7 ± 0.7	14.0 ± 0.8	12.4 ± 0.7	12.9 ± 0.6
Rmax	39.1 ± 1.8	37.8 ± 3.5	40.4 ± 1.7	40.0 ± 2.2

Data are expressed as mean ± SEM.

The intracellular concentration of fura-2, the leakage of this dye, and Rmax, the index of hydrolysis of the fluorescent indicator, were similar in the two groups, and were unaffected by manipulation of the salt content of the diet (Table 2). Thus, we are able to reliably compare the value of $[Ca^{2+}]i$ determined in fura-2 loaded platelets between the groups and dietary periods.

During the low salt diet, the basal platelet $[Ca^{2+}]i$ was similar in both the SS and SR patients. The intake of a high NaCl diet for 1 week increased the basal $[Ca^{2+}]i$ in both groups regardless of the salt sensitivity of their blood pressures (SS, from 22.0 ± 1.3 nM to 27.2 ± 1.9 nM, $p<0.01$; SR, from 20.1 ± 0.8 nM to 24.4 ± 1.3 nM, $p<0.05$. Fig. 1). The magnitude of the increase in basal $[Ca^{2+}]i$ of the SS patients was not different from that of the SR patients. There was no difference between SS and SR in the maximal Ca^{2+} response to thrombin in the range of 0.03～1.0 U/ml in the presence of extracellular Ca^{2+} during the low salt diet period (Fig. 2). High NaCl loading did not change the thrombin induced Ca^{2+} response in the presence of extracellular Ca^{2+} either in SS or SR. Increases in $[Ca^{2+}]i$ in the absence of extracellular Ca^{2+} in response to thrombin were also similar between SS and SR during both the low and high salt diets (Fig. 3). Sampled 5 points of $[Ca^{2+}]i$ after the peak response to thrombin in the absence of extracellular Ca^{2+} were well fitted to the equation: $[Ca^{2+}]i = Ae^{-kt}$ (r value was more than 0.8 in each experiment). During the low salt diet period, the value of A in SS patients was similar to that in SR (SS, 100.9 ± 10.4 nM; SR, 125.5 ± 14.8 nM). The k value, which represents the rate constant of $[Ca^{2+}]i$ recovery, tended to be larger in SR than in SS (SS, 0.005 ± 0.001 sec^{-1}; SR, 0.024 ± 0.012 sec^{-1}, $p=0.08$, Fig. 4). The value of A was not changed by salt loading either in the SS or SR patients (SS, 100.8 ± 15.4 nM; SR, 109.6 ± 12.7 nM). During the high salt diet period, the k value in SR was significantly larger than that in SS (SS, 0.004 ± 0.001 sec^{-1}; SR, 0.043 ± 0.014 sec^{-1}, $p<0.05$).

The intracellular Ca^{2+} discharge capacity during the low salt diet was similar in both groups. (SS, 658.1 ± 52.8 nM; SR, 690.8 ± 65.1 nM, Fig. 5) However, excessive intake of NaCl significantly increased the intracellular Ca^{2+} discharge capacity in SR patients but caused no change in the SS

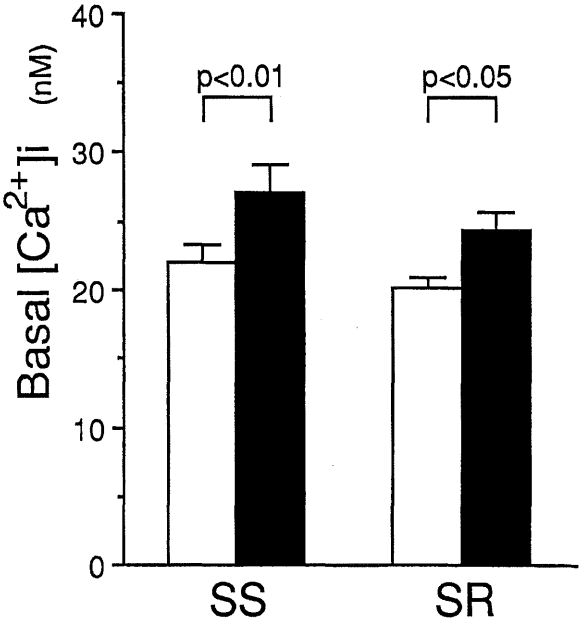

Fig. 1. Platelet basal $[Ca^{2+}]i$ during low and high salt diet periods.
There was no difference in basal $[Ca^{2+}]i$ between SS and SR patients during the low salt diet period (open bar) and high salt diet period (closed bar). Basal $[Ca^{2+}]i$ significantly increased by dietary salt loading and the magnitude of change in $[Ca^{2+}]i$ was similar in both groups.

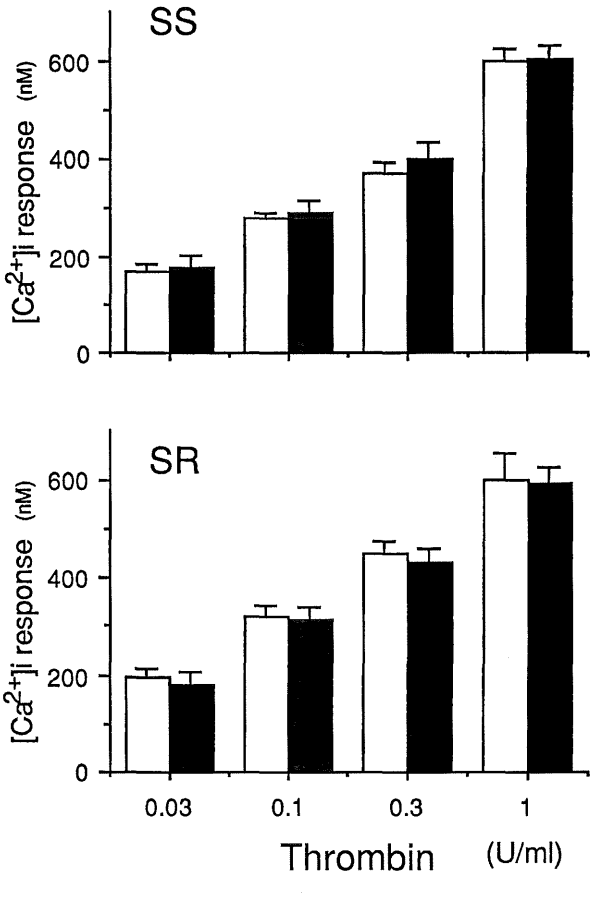

Fig. 2. Thrombin induced $[Ca^{2+}]i$ response in the presence of 1 mM of extracellular Ca^{2+} during low and high salt diet periods.
There was no difference in $[Ca^{2+}]i$ response to thrombin in the range of 0.03～1.0 U/ml between SS (upper panel) and SR (lower panel) during the low salt diet period (open bar). $[Ca^{2+}]i$ responses during the high salt diet period (closed bar) did not altere from those during the low salt period.

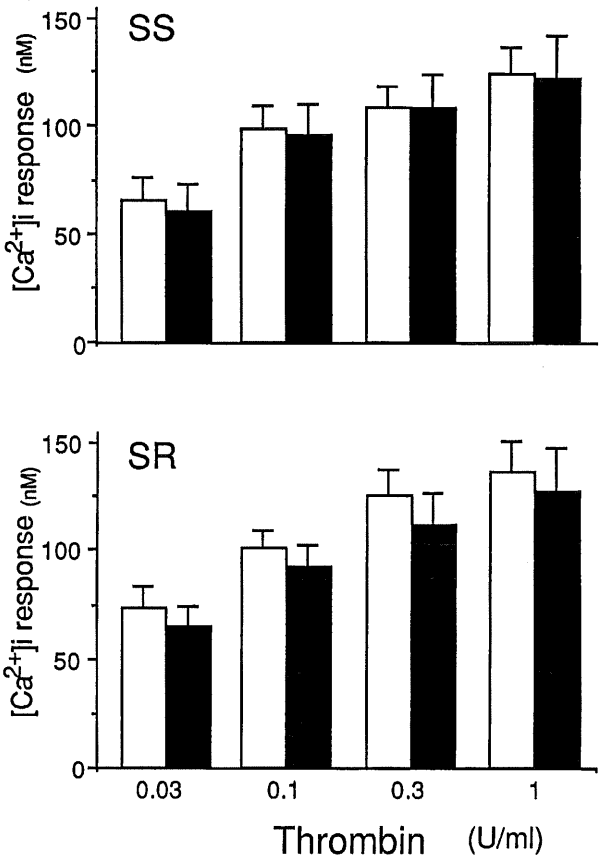

Fig. 3. Thrombin induced $[Ca^{2+}]i$ response in the absence of extracellular Ca^{2+} during low and high salt diet periods.
There was no difference in $[Ca^{2+}]i$ response to thrombin in the range of 0.03～1.0 U/ml between SS (upper panel) and SR (lower panel) during the low salt diet period (open bar). Excessive salt intake did not alter the $[Ca^{2+}]i$ response either in SS or SR patients. (closed bar)

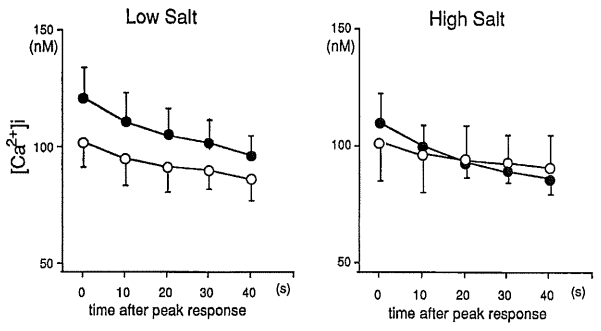

Fig. 4. Decay in $[Ca^{2+}]i$ after peak response to 0.3 U/ml of thrombin.
The curve of decrease in $[Ca^{2+}]i$ was fitted to the equation: $[Ca^{2+}]i=Ae^{-kt}$. The rate constant (k) of SR (closed circle) during the low salt diet period tended to be larger than that of SS (open circle, left panel). During the high salt diet period, the rate constant of SR was significantly larger than that of SS patients (right panel, $p<0.05$).

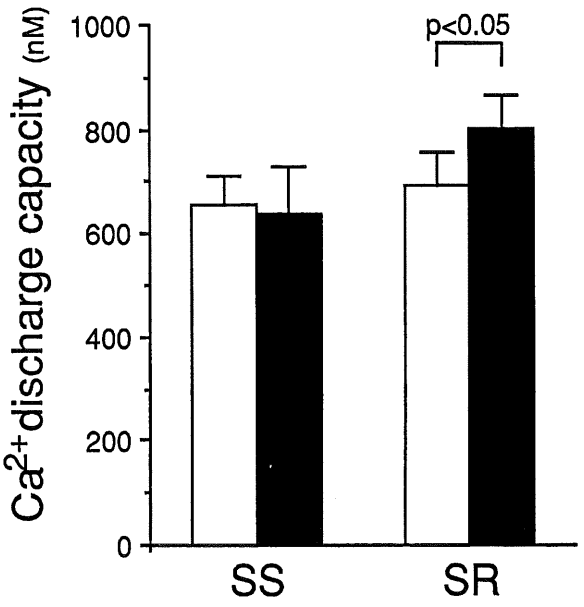

Fig. 5. Platelet intracellular Ca^{2+} discharge capacity assessed by 5 μM of ionomycin during low and high salt diet periods.
There was no difference in Ca^{2+} discharge capacity between SS and SR patients during the low salt diet period (open bar). Although excessive salt intake did not alter Ca^{2+} discharge capacity in SS patients, dietary salt loading significantly increased Ca^{2+} discharge capacity in SR patients (closed bar).

patients (SS; 639.6±91.9 nM; SR; 803.3±65.1 nM).

DISCUSSION

Cellular Ca^{2+} is one of the important second messengers and plays a key role in cellular functions including the contraction of vascular smooth muscle cells[20], the secretion of endocrine hormones[2], and the aggregation and secretion of platelets[29]. Thus abnormalities in the cellular metabolism of Ca^{2+} can lead to clinical disorders. Modifications in Ca^{2+} handling and other electrolyte metabolism[30,31] have been reported in vascular smooth muscle cells[22,36] and lymphocytes[24] in hypertension. Basal $[Ca^{2+}]i$ in platelets was also reported to increase both in patients with essential hypertension[6] and in spontaneously hypertensive rats[27]. In addition, enhanced Ca^{2+} responsiveness after stimulation with arginine vasopressin has been observed in hypertension[19] and an increase in $[Ca^{2+}]i$ is considered to be responsible, at least in part, for its development[6].

We tested the ability of salt loading to increase basal $[Ca^{2+}]i$ in the platelets of patients with essential hypertension. A circulating plasma factor isolated from patients with essential hypertension has been reported to increase basal $[Ca^{2+}]i$ and to enhance the Ca^{2+} response in plate-

lets[12,18]. Such a digitalis-like factor alters cellular Ca^{2+} handling by vascular smooth muscle cells[8] and platelets[23]. Because a high salt intake may elevate the circulating levels of endogenous digitalis-like factor(s)[10], these molecules may be involved in the elevation in the platelet $[Ca^{2+}]i$ induced by salt loading. However, in the present study, basal $[Ca^{2+}]i$ increased in both SS and SR groups regardless of salt sensitivity. The change in basal $[Ca^{2+}]i$ did not differ between the SS and SR patients. Therefore, the change in basal $[Ca^{2+}]i$ after salt loading may not be a major determinant of salt sensitivity. However, we cannot exclude the possibility that an increase in basal $[Ca^{2+}]i$ could be the initial trigger in the pressor response to salt loading, and the compensatory mechanisms are at work. It is possible that elevations in blood pressure following salt loading are due to insufficient compensatory mechanisms.

Our finding that the change in basal $[Ca^{2+}]i$ by salt loading did not differ between the SS and SR patients is inconsistent with earlier reports that indicated that a significant increase in basal $[Ca^{2+}]i$ was observed only in SS patients[1,28]. The reason for this discrepancy is unclear. However, the cellular metabolism of Ca^{2+}-sensitive fluorescent dye, especially dye leakage, has not been considered in previous reports[1,25,28,34]. We demonstrated that fura-2 metabolisms did not differ between the SS and SR patients and that dietary salt manipulation had no effect on dye hydrolysis or leakage in either group. If correction for dye leakage is not performed, the $[Ca^{2+}]i$ would be misestimated because there is a vast Ca^{2+} gradient between the extra- and intracellular space. Failure to correct for dye leakage could thus lead to an incorrect interpretation[27].

It is well known that excessive salt intake enhances platelet aggregation which causes cardiovascular events accompanied by an elevation in blood pressure[21]. However, an enhancement of platelet aggregation and secretion after salt loading was reported to be independent of changes in blood pressure in SR hypertensive patients[35]. The elevation of platelet basal $[Ca^{2+}]i$ by excessive salt intake could contribute, in part, to enhanced platelet aggregation despite the lack of blood pressure elevation in SR patients.

We previously reported that the lymphocyte level of $[Ca^{2+}]i$ is correlated with the hypotensive responses to nifedipine in patients with essential hypertension[25]. Zemel et al[37] demonstrated that the exaggerated response of platelet Ca^{2+} flux to arginine vasopressin could be used to predict the development of preeclampsia. Thus, the concentration of Ca^{2+} in circulating blood cells could be a useful clinical marker. However, no difference in basal $[Ca^{2+}]i$, maximal Ca^{2+} responses irrespective of extracellular Ca^{2+}, and the intracellular Ca^{2+} discharge capacity between SS and SR patients were seen during the low salt diet. These indices of platelet Ca^{2+} handling during a low salt diet period failed to predict salt sensitivity in patients with essential hypertension.

The decline in $[Ca^{2+}]i$ after the peak response to thrombin was considered as the sum of Ca^{2+} uptake to salcoplasmic reticulum and Ca^{2+} extrusion to extracellular space via adenosine triphosphate-driven Ca^{2+} pumps[33] and Na^{+}-Ca^{2+} exchange[15]. The rate of decay of the Ca^{2+} transient in the Ca^{2+} free medium was greater in SR than SS and the difference in the rate constant of Ca^{2+} recovery between SS and SR was augmented by dietary salt loading. Although the maximal $[Ca^{2+}]i$ response in SR was similar to that in SS during the high salt diet period, the rapid recovery of $[Ca^{2+}]i$ after stimulation may be involved, in part, in preventing blood pressure elevation after salt loading in SR patients.

This study is the first to demonstrate the effect of dietary salt intake on the intraplatelet Ca^{2+} discharge capacity. Because the intracellular Ca^{2+} discharge capacity, assessed by an adequate amount of ionomycin, was regarded as the intracellular Ca^{2+} storage size, the ionomycin sensitive intracellular Ca^{2+} storage size was increased in SR patients after salt loading. However, $[Ca^{2+}]i$ increases in response to thrombin in the absence of extracellular Ca^{2+}, which was considered as Ca^{2+} release from intracellular Ca^{2+} storage sites, were unaltered by dietary salt loading in SR patients. This study indicated that dietary salt loading increases the thrombin-insensitive intracellular Ca^{2+} storage component in SR patients. These results suggested that the ionomycin sensitive Ca^{2+} storage size in SR patients may operate as a Ca^{2+} buffering capacity. Furthermore, it has been demonstrated that intracellular Ca^{2+} storage size modulates the cellular Ca^{2+} metabolism such as Ca^{2+} uptake, inositol 1,4,5-triphosphate mediated Ca^{2+} release, Ca^{2+} induced Ca^{2+} release, and store-regulated Ca^{2+} entry in human platelets[11]. It is possible that the increased Ca^{2+} storage size may affect Ca^{2+} responsiveness to other agonists than thrombin and may participate in the difference in Ca^{2+} handling between SS and SR patients after salt loading. An increased intracellular Ca^{2+} storage size may be related to enhanced Ca^{2+} extrusion from cytosol in SR patients during a high salt diet period. Therefore, the increase in intracellular Ca^{2+} discharge capacity in SR patients might play a significant role, in part, in the salt resistance of blood pressure through the increase in Ca^{2+} buffering capacity and the alteration of Ca^{2+} handling. As the heterogeneity in intracellular Ca^{2+} stores has been indicated, further studies are necessary to clarify which component of the Ca^{2+} store is modified by dietary salt intake.

In conclusion, no index of platelet Ca^{2+} handling during a low salt diet period could predict the salt sensitivity in patients with essential hypertension. Salt loading resulted in increased basal $[Ca^{2+}]i$ both in the SS and SR patients. However, because the magnitude of change in basal $[Ca^{2+}]i$ was similar in both groups, the increment in basal $[Ca^{2+}]i$ may not be a major determinant in the pressor response to salt loading. Although maximal $[Ca^{2+}]i$ response to thrombin was not changed by a high salt intake either in the SS or SR patients, the rate of $[Ca^{2+}]i$ recovery after the peak response to thrombin was larger in SR than SS during the high salt diet period. Dietary salt loading increased intraplatelet Ca^{2+} storage size in the SR patients but it was unchanged in the SS patients. An increased intracellular Ca^{2+} storage size in SR patients may contribute, at least in part, to the salt resistance of blood pressure.

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