Intralymphocytic Sodium and Free Calcium and Plasma Renin in Essential Hypertension

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SUMMARY Intracellular sodium, potassium, and free calcium concentrations were investigated in lymphocytes of 30 patients with essential hypertension and 30 normotensive controls. All subjects were placed on a diet containing 8 to 10 g of sodium chloride per day. Lymphocyte sodium concentration was higher in hypertensive patients than in normotensive controls (19.8 ± 1.8 vs 18.4 ± 1.8 mmol/kg wet weight; \( p < 0.01 \)), whereas lymphocyte potassium concentration was similar in both groups. Lymphocyte free calcium concentration was also higher in hypertensive patients than in normotensive controls (134.6 ± 13.2 vs 120.2 ± 16.4 nmol/L; \( p < 0.01 \)). There was a positive correlation between lymphocyte sodium and free calcium concentrations in normotensive controls, in hypertensive patients, and in the subjects combined (\( r = 0.59, p < 0.01; r = 0.71, p < 0.001; \) and \( r = 0.70, p < 0.001, \) respectively). Lymphocyte potassium concentration was not related to lymphocyte sodium or free calcium concentration in each group. In patients with essential hypertension, intracellular sodium and free calcium concentrations were negatively correlated with plasma renin activity (\( r = -0.66, p < 0.001; r = -0.60, p < 0.001, \) respectively), but they were not related to age, mean blood pressure, serum electrolyte concentration, or plasma norepinephrine concentration. These results suggest that a considerable relationship exists between intracellular sodium and free calcium in lymphocytes and that, in essential hypertension, the alteration in cellular metabolism of sodium and calcium may be linked to the renin system but not to blood pressure, age, or adrenergic activity. (Hypertension 12: 26–31, 1988)

KEY WORDS • intracellular free calcium concentration • intracellular sodium concentration • plasma renin activity • lymphocytes • essential hypertension

In recent decades, there has been increasing evidence of an abnormality of sodium transport across the cell membrane associated with elevated intracellular sodium concentration ([Na\(_i\)]) in erythrocytes, leukocytes, and lymphocytes of patients with essential hypertension. Alternatively, abnormal calcium handling and increased intracellular free calcium concentration ([Ca\(^{2+}\)]) have been reported in erythrocytes and platelets of patients with essential hypertension. The increases in [Na\(_i\)] and [Ca\(^{2+}\)] are considered, at least in part, to play an important role in the pathogenesis of essential hypertension. Although a link between sodium transport and calcium handling at the cellular level has been postulated, only limited information is available concerning the relation between abnormalities in [Na\(_i\)] and [Ca\(^{2+}\)], in patients with essential hypertension.

An abnormality of cellular cations may be demonstrated only in a subgroup of and not in all patients with essential hypertension because of the pathogenetic heterogeneity of the hypertensive populations. If elevations of [Na\(_i\)] and [Ca\(^{2+}\)] can be detected in essential hypertension, it is important to assess the factors determining such alterations in cation metabolism at the cellular level. Thus, in the present study, [Na\(_i\)], intracellular potassium concentration ([K\(_i\)]), and [Ca\(^{2+}\)], were determined simultaneously in lymphocytes of patients with essential hypertension to elucidate the relation of [Na\(_i\)], [K\(_i\)], and [Ca\(^{2+}\)], to each other and to several clinical indices such as age, blood pressure,
serum electrolyte concentration, plasma renin activity, and plasma norepinephrine concentration.

Subjects and Methods

Thirty outpatients with essential hypertension and 30 normotensive controls were studied. All subjects were Japanese. No subjects had taken antihypertensive drugs or any other drugs for 4 weeks before the study. All the patients with essential hypertension had systolic blood pressure of more than 160 mm Hg or diastolic blood pressure of more than 95 mm Hg in the sitting position (or both) on at least three occasions in the outpatient clinic. All the normotensive controls had systolic blood pressure of less than 140 mm Hg and diastolic blood pressure of less than 90 mm Hg when screened. Patients with secondary causes of hypertension and with extensive organ damage were excluded on the basis of appropriate clinical, biochemical, and radiological examinations. After the nature and purpose of the study were explained, informed consent was obtained from each subject.

All of the subjects were on a diet containing 8 to 10 g of sodium chloride per day for 1 week before the day of the study. During that week, 24-hour urine samples were used to determine the excretion of sodium, potassium, calcium, and creatinine as a check on dietary adherence. The mean of these determinations was considered a measure of intake.

Subjects fasted overnight, and a catheter then was inserted in the forearm vein and kept patent with 5% glucose to obtain blood samples. After the subjects remained supine for at least 30 minutes in a quiet and dark room, blood pressure was measured with a mercury sphygmomanometer every minute for 10 minutes. The average of 10 consecutive blood pressure measurements was used in the analyses. Mean blood pressure was calculated as diastolic blood pressure plus one third of pulse pressure. Subsequently, 45 ml of blood was obtained for blood pressure plus one third of pulse pressure. Mean blood pressure was calculated as diastolic pressure measurements was used in the analyses. Serum and urinary sodium, potassium, calcium, and creatinine concentrations; plasma renin activity; plasma norepinephrine concentration; and [Na], [K], and [Ca2+] in lymphocytes. Serum and urinary sodium, potassium, calcium, and creatinine concentrations were measured by routine chemical methods. Plasma renin activity was measured by a radioimmunoassay method,12 and plasma norepinephrine concentration was measured by an electrochemical method using high performance liquid chromatography.13

Isolation of lymphocytes was performed with a modification of Boyum's method.14 It consists of diluting heparinized blood 1:2 with medium RPMI-1640 (Gibco Laboratories, Grand Island, New York, USA), layering on a Ficoll-metrizoate mixture (Lymphoprep, Nyegaard, Oslo, Norway) with a density of 1.077 g/L, and centrifuging at 400 g for 10 minutes and prepared for measurements of [Na], [K], and [Ca2+].

[Na], and [K], in lymphocytes were measured by a modification of the method of Ambrosioni et al.3 In brief, the cells were washed twice with cold isotonic MgCl2 solution, transferred to a polycarbonate capillary tube (Hematolon, Kayagaki Irinkogyo, Tokyo, Japan), and centrifuged at 15,000 g for 5 minutes. When the lymphocytes became packed at the bottom of the tube, the tube was cut at the boundary between the packed lymphocytes and supernatant. The percentage of extracellular fluid volume trapped within the lymphocyte pellet determined by 131I-labeled human serum albumin (Commission a L'Energie Atomique, Paris, France) was about 26%. The cells packed in the tube were then weighed and dried out at 80 °C for 48 hours. Nitric acid (14 N) was then added, and the cellular pellet was completely dissolved. The empty capillary tubes were then weighed. Thus, 74% of the weight loss equaled the wet weight of the lymphocytes. [Na], and [K], in lymphocytes were determined by flame photometry (Model 775-A, Hitachi, Tokyo, Japan) and expressed as millimoles per kilogram of wet weight. The intra-assay coefficient of variation was less than 5%, and the day-to-day intrasubject coefficient of variation was less than 7% for [Na]. Corresponding coefficients of variation for [K], were less than 6% and less than 9%, respectively. [Ca2+] in lymphocytes was determined by a modification of the method of Tsien et al.15 Briefly, the lymphocytes (1–3 × 107/ml) were incubated at 37 °C for 40 minutes with 50 μM of the fluorescent dye, quin 2 tetraacetoxymethylester (quin 2 AM, Dojindo Laboratories, Kumamoto, Japan). After loading with quin 2 AM, the cells were washed, transferred to fresh RPMI-1640, and left at room temperature for 60 minutes to allow hydrolysis of the ester. The evidence that loaded lymphocytes maintain a high eosin exclusion (>95%) weighed against gross toxicity of the quin 2 concentration in the present study. The lymphocytes were centrifuged at 400 g for 10 minutes and resuspended (3–5 × 106 cells/ml) in physiological saline containing (mM) NaCl, 145; KCl, 5; HEPES, 10; Na2HPO4, 1; CaCl2, 1; MgSO4, 0.5; and glucose, 5, at pH 7.4 and 37 °C. Fluorescence was measured at 37 °C with a Hitachi spectrofluorimeter (Model 204 S, Tokyo, Japan). Excitation and emission wavelengths were 339 and 492 nm with 4- and 10-nm bandwidth, respectively. Resting levels of [Ca2+] in lymphocytes were calculated by the equation [Ca2+] = 115 nM × (F/ [F]max) ([F]max = F), where 115 nM is the effective dissociation constant (Kd) of the calcium-quin 2 complex and F is the fluorescence of the intact cell suspension. [F]max is the fluorescence measured by releasing the dye from the cells with digitonin, and Fmin is that measured after adjustment of pH to about 8.5 by adding Tris and setting extracellular calcium concentration to 1 nM by adding 2 mM EGTA. The values of F, [F]max, and Fmin were corrected for the
TABLE 1. Clinical Characteristics of Hypertensive Patients and Normotensive Controls

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotensive</th>
<th>Hypertensive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex (M/F)</td>
<td>16:14</td>
<td>14:16</td>
</tr>
<tr>
<td>Age (yr)</td>
<td>52.5±15.3</td>
<td>52.5±10.8</td>
</tr>
<tr>
<td>Mean blood pressure (mm Hg)</td>
<td>85.2±10.4</td>
<td>114.1±10.5*</td>
</tr>
</tbody>
</table>

**Serum**
- Sodium (mmol/L): 142.0±3.6 for normotensive, 114.1±10.5* for hypertensive
- Potassium (mmol/L): 2.32±0.09 for normotensive, 4.22±0.46 for hypertensive
- Calcium (mmol/L): 0.96±0.09 for normotensive, 2.31±0.08 for hypertensive
- Creatinine (mg/dl): 4.22±0.39 for normotensive, 2.32±0.09 for hypertensive
- Plasma renin activity (ng Ang I/ml/hour): 2.26±1.28 for normotensive, 154±64 for hypertensive
- Norepinephrine (pg/ml): 138±74 for normotensive, 1238±189 for hypertensive

**Plasma**
- Plasma renin activity (ng Ang I/ml/hour): 2.26±1.28 for normotensive, 154±64 for hypertensive
- Norepinephrine (pg/ml): 138±74 for normotensive, 1238±189 for hypertensive

**Urine volume (ml/day)**: 1238±189 for normotensive, 1212±265 for hypertensive

**Urinary excretion**
- Sodium (mmol/day): 156.0±10.9 for normotensive, 151.4±10.2 for hypertensive
- Potassium (mmol/day): 50.6±8.4 for normotensive, 48.8±9.8 for hypertensive
- Calcium (mmol/day): 3.45±0.07 for normotensive, 2.30±0.08 for hypertensive
- Creatinine (g/day): 1.12±0.11 for normotensive, 1.22±0.13 for hypertensive

Values are means ± SD. Ang I = angiotensin I.

*p < 0.05, compared with values in normotensive controls.

Autofluorescence of the cell before and after cell lysis and of added reagents such as EGTA. The intra-assay coefficient of variation was less than 7%, and the day-to-day intrasubject coefficient of variation was less than 8%.

Data are presented as means ± SD. Statistical analyses were performed using Wilcoxon rank-sum test to compare the different study groups. Results were considered significant if the p value was less than 0.05. Correlations between parameters were tested by linear regression analysis.

**Results**

Age; sex; serum sodium, potassium, calcium, and creatinine concentrations; plasma renin activity; plasma norepinephrine concentration; and urinary excretion of sodium, potassium, calcium, and creatinine did not differ between normal controls and hypertensive patients (Table 1). The individual variation of the urinary excretion of sodium, potassium, and calcium was small and did not affect lymphocyte [Na]i, [K]i, or [Ca2+]i, in either group.

Intracellular cation concentrations in lymphocytes of hypertensive patients in comparison with those of normotensive controls are shown in Figure 1. [Na]i in lymphocytes was significantly higher (p < 0.01) in hypertensive patients (19.8 ± 1.8 mmol/kg wet weight) than in normotensive controls (18.4 ± 1.8 mmol/kg wet weight), while [K]i in lymphocytes of hypertensive patients (99.9 ± 6.0 mmol/kg wet weight) did not differ from that of normotensive controls (99.6 ± 5.8 mmol/kg wet weight). [Ca2+]i in lymphocytes was also significantly higher (p < 0.01) in hypertensive patients (134.6 ± 13.2 nmol/L) than in normotensive controls (120.2 ± 16.4 nmol/L).

The relationship between [Na]i and [Ca2+]i in lymphocytes is shown in Figure 2. A close linear correlation was present in normotensive controls (r = 0.59, p < 0.01), in hypertensive patients (r = 0.71, p < 0.001), and in the group combined (r = 0.70, p < 0.001), respectively. [K]i had no relation to [Na]i or [Ca2+]i, in each group.

As shown in Figure 3, plasma renin activity was negatively correlated with lymphocyte [Na]i (r = -0.66, p < 0.001) and [Ca2+]i (r = -0.60, p < 0.001) in the hypertensive patients. No correlation could be demonstrated between plasma renin activity and [Na]i or [Ca2+]i in normotensive controls. Age had a statistically significant correlation with lymphocyte [Na]i and [Ca2+]i in normotensive controls (r = 0.44, p < 0.05; r = 0.51, p < 0.01, respectively), but not in hypertensive patients (r = 0.31, NS; r = 0.25, NS, respectively). Mean blood pressure was correlated with neither [Na]i nor [Ca2+]i in normotensive controls.
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\( r = 0.23, \text{NS for } [\text{Na}]_i; r = 0.13, \text{NS for } [\text{Ca}^{2+}]_i \) and in hypertensive patients \( r = 0.20, \text{NS for } [\text{Na}]_i; r = 0.11, \text{NS for } [\text{Ca}^{2+}]_i \). There was no relation between intracellular sodium, potassium, or free calcium concentration and serum sodium, potassium, or calcium concentration or plasma norepinephrine level in each group.

**Discussion**

An elevation of [Na], has been reported on a number of occasions in erythrocytes, leukocytes, and lymphocytes of patients with essential hypertension, while [K], is considered to be unaltered in such blood cell types of this disease. The increased [Na], in essential hypertension has been explained by the sodium transport hypothesis and supported by multiple experimental observations, beginning with Dahl et al. and followed by Haddy et al., de Wardener and MacGregor, and many others. The hypothesis is that essential hypertension is due to a rise in plasma concentration of a circulating Na⁺,K⁺-adenosine triphosphatase (ATPase) inhibitor secreted in response to volume expansion. Na⁺, K⁺-ATPase inhibitor increases [Na], through a reduction in sodium efflux. A Na⁺-Ca²⁺ exchange system has been assumed to participate in the regulation of calcium handling of a number of cells. Increased [Na], would decrease the rate of extracellular Na⁺-dependent Ca²⁺ efflux and increase the rate of intracellular Na⁺-dependent Ca²⁺ influx and thereby raise [Ca²⁺], and thus increase vascular reactivity. This Na⁺-Ca²⁺ exchange has been demonstrated in vascular smooth muscle cells by Blaustein.

Since the factors regulating blood pressure are complex and essential hypertension should not be regarded as a single disease entity, many investigators have separated this disease into distinct subgroups with its own characteristics. Because the distribution of plasma renin activity in patients with essential hypertension shows a continuous pattern, the division of patients into low, normal, and high renin subgroups may be arbitrary. However, many investigators have emphasized the uniqueness of patients with low renin hypertension, reporting that they have a different mechanism for their hypertension and an enhanced response to diuretics. Accordingly, it is suggested that, in these patients with low renin activity, the plasma volume is effectively expanded through a greater tendency of the kidneys to retain sodium compared with patients with normal or high renin activity. If this suggestion is correct, the abnormality in [Na], should be greatest in patients with low renin hypertension according to the sodium transport hypothesis that a circulating Na⁺,K⁺-ATPase inhibitor is stimulated by volume expansion. In fact, Na⁺,K⁺-ATPase-dependent sodium efflux from leukocytes was reported to be smallest in patients with low renin hypertension. In the present study, in patients with essential hypertension, both [Na], and [Ca²⁺], were increased and negatively correlated with plasma renin activity. Our findings may support the sodium transport hypothesis; however, it is difficult to decide whether lymphocytes possess a Na⁺-Ca²⁺ exchange mechanism. Some investigators have failed to demonstrate the existence of Na⁺-Ca²⁺ exchange. Using quin 2, Tsien et al. reported that [Ca²⁺], in lymphocytes was unchanged with changes in extracellular sodium concentration, and Shore et al. and Bruschi et al. reported that incubation with ouabain did not affect [Ca²⁺], in lymphocytes. These observations may result from an inhibitory effect of quin 2 on Na⁺-Ca²⁺ exchange. The data on Na⁺-Ca²⁺ exchange in lymphocytes are limited by the single report of Ueda on Na⁺-dependent Ca²⁺ fluxes in membrane vesicles prepared from rabbit lymphocytes. The uptake of Ca²⁺ through this Na⁺...
was observed between [Na] and [Ca\textsuperscript{2+}], in both hypertensive patients and normotensive controls. We also observed that [Ca\textsuperscript{2+}]\textsubscript{i} in lymphocytes was higher in patients with essential hypertension than in normotensive controls and had a negative correlation with plasma renin activity. These results are in accord with the results of studies on the response to calcium antagonists. The hypotensive effects of calcium antagonists also have been demonstrated to be enhanced in patients with essential hypertension compared with normotensive subjects and negatively correlated with plasma renin activity. If the hypotensive effects of these drugs reflect a functional abnormality of vascular smooth muscle related to [Ca\textsuperscript{2+}], lymphocytes may be an appropriate model for calcium handling in vascular smooth muscle cells in essential hypertension.

In summary, [Na], and [Ca\textsuperscript{2+}]\textsubscript{i}, in lymphocytes were higher in hypertensive patients than in normotensive controls. There was a positive correlation between [Na], and [Ca\textsuperscript{2+}], in hypertensive patients and normotensive controls. Plasma renin activity was negatively correlated with [Na], and [Ca\textsuperscript{2+}], in hypertensive patients. We could not find a significant association of age, blood pressure, or plasma norepinephrine concentration with these alterations in cellular cation. These results suggest that the elevation in [Ca\textsuperscript{2+}], linked with increased [Na], is important in the genesis of volume-expanded hypertension with suppressed plasma renin activity.
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