Leukocyte Ionized Calcium and Sodium Content and Blood Pressure in Humans

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SUMMARY The relationship between leukocyte ionized calcium concentration, sodium content, and blood pressure was studied in normotensive subjects with (n = 17) and without (n = 21) a family history of hypertension and in untreated patients with essential hypertension (n = 22). There was a highly significant correlation between intracellular ionized calcium and mean supine blood pressure (measured on the same occasion) in normal subjects with no family history of hypertension (r = +0.73, p < 0.01). This relationship was lost in normal subjects with a family history of hypertension, and in hypertensive patients there was a nonsignificant negative correlation between intracellular ionized calcium and blood pressure (r = +0.08 and −0.31, respectively). Intracellular ionized calcium was similar in the normotensive groups (both, 126 ± 7 nmol/L) and slightly but nonsignificantly elevated in hypertensive patients (143 ± 10 nmol/L; p = 0.09). There was no correlation between intracellular ionized calcium and sodium content in any group (r < 0.1). These results indicate that while leukocyte ionized calcium in normotensive subjects with no family history of hypertension may reflect smooth muscle contractility resulting in the positive correlation between leukocyte ionized calcium and blood pressure, this relationship is lost in hypertensive patients and subjects predisposed to hypertension. This may be due to an altered relationship between leukocyte and smooth muscle calcium handling in these subjects or to non-calcium-mediated influences on blood pressure. (Hypertension 8: 483-488, 1986)

KEY WORDS • cytosolic calcium • sodium • leukocyte • blood pressure

INTRACELLULAR calcium is important in the regulation of several cellular functions, including smooth muscle contraction.1,2 In essential hypertension cellular handling of calcium has been reported to be abnormal in blood cells and adipose tissue,3 and similar abnormalities have been shown in the spontaneously hypertensive rat, including vascular smooth muscle in this model.3-5 In addition, an enhanced vasodilator response to calcium channel blockers has been demonstrated in essential hypertension, supporting the view that cellular calcium homeostasis is disturbed.6,7 These abnormalities in cellular calcium handling could directly result in an increase in intracellular ionized calcium. Alternatively, increased cell sodium, which has been reported in blood cells in essential hypertension,8,9 could produce a rise in intracellular calcium through a Na⁺-Ca²⁺ exchange process.10

Recently, increased intracellular calcium levels in essential hypertension have been reported in erythrocytes, using ion-selective electrodes,11,12 and in platelets, using the fluorescent indicator quin 2.13 In one of the latter studies13 a close relationship between intracellular ionized calcium and blood pressure was found in both normotensive and hypertensive populations. No significant increase in intracellular calcium has been shown in white blood cells using similar techniques,14,15 although it is in the leukocyte that abnormalities in sodium handling have been reported in hypertensive patients and normotensive subjects with a family history of hypertension.16-18

We have studied the relationships between intracellular calcium and sodium content in white blood cells and blood pressure in normotensive subjects with and without a family history of hypertension as well as in a group of patients with untreated essential hypertension.
Subjects and Methods
Twenty-two hypertensive patients were seen in the Leicester Hypertension Clinic. All had diastolic blood pressures greater than 90 mm Hg (phase V) on at least two occasions, had not previously received treatment for hypertension, and had no evidence of a cause for the hypertension.

Normotensive subjects were recruited from the general population and had a diastolic blood pressure of less than 90 mm Hg when screened. They were prospectively selected according to whether or not they had a family history of hypertension. A positive family history was defined as at least one parent or sibling who was receiving treatment for hypertension or who had died from a complication of hypertension; 17 subjects with a family history and 21 without a family history of hypertension were studied.

All subjects were seen between 0900 and 1200 on the study day. Each subject’s blood pressure was recorded after 15 minutes supine and 2 minutes standing using a Hawksley random zero sphygmomanometer (Lancing, Sussex, England), diastolic phase V, and blood was then taken for measurement of intracellular ionized calcium ([Ca\(^{2+}\)]\(_i\)) and sodium content ([Na]\(_i\)) in white blood cells. All subjects studied were white, no subject was taking any medication, and diet was unrestricted. All subjects gave full, informed consent.

A suspension of white blood cells was prepared as previously described. Venous blood anticoagulated with heparin was mixed with Plasmagel (Uniscience, London, UK; ratio, 2:1), and after standing for 30 minutes at 37°C, the supernatant was aspirated and centrifuged (300 g for 10 minutes at 37°C). Any remaining erythrocytes were removed by hypotonic lysis. Recoveries were around 50% with a normal differential cell count (70% neutrophils, 18% lymphocytes, 3% eosinophils and basophils, and 9% unclassifiable cells) and morphology.

To obtain [Ca\(^{2+}\)], an aliquot of the white blood cell suspension was loaded with quin 2 according to the method of Tsien et al. Cells (10\(^5\)/ml) were incubated with 20 \(\mu\)M quin 2 acetoxymethyl ester (Lancaster Laboratories, Morecombe, UK) for 20 minutes at 37°C in a physiological buffer containing (in mM) NaCl, 145; KCl, 5; Na\(_2\)HPO\(_4\), 1; MgSO\(_4\), 0.5; glucose, 5; N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), 10; CaCl\(_2\), 1; and 0.5% bovine serum albumin. After dilution (\(\times\) 10) with buffer the incubation was continued for a further 90 minutes. The cells were then centrifuged and resuspended in fresh buffer. Aliquots were centrifuged and resuspended in buffer without bovine serum albumin, and fluorescence was measured immediately in a Perkin-Elmer LS-3 fluorimeter (excitation, 340 nm; emission, 495 nm; Norwalk, CT, USA) with the cuvettes maintained at 37°C. Maximum fluorescence in 1 mM calcium was measured 10 minutes after the addition of the ionophore A23187 (2 \(\mu\)M; Sigma Chemical Co., Poole, UK), and minimum fluorescence was measured after the addition of 13 mM ethylene glycol bis(\(\beta\)-aminoethyl ether)-\(N,N,N',N'\)-tetraacetic acid and 0.2% Triton X-100 and adjustment of the pH to greater than 8.4. An aliquot of the original cell suspension treated in an identical manner but with the addition of quin 2 acted as a control for the autofluorescence of the cell suspension and additions such as the ionophore and Triton X-100. Intracellular calcium (nmol/L) was calculated from the following equation after the readings for quin-loaded cells had been corrected for control values: \([\text{Ca}^{2+}] = 115 \times \frac{(F_m - F_m^\text{min})}{(F_m^\text{max} - F_m)}\), where 115 is the dissociation constant for quin 2 under these conditions and \(F_m\) is the fluorescence of the sample. Measurements were made in triplicate. The intraassay coefficient of variation was 8.1%, and the day-to-day individual variation was 12.1%.

To obtain [Na], an aliquot of the original white blood cell suspension was incubated in buffer for 30 minutes at 37°C. After being washed twice with isotonic magnesium chloride (99 mM, 4°C), the pellet was placed in a preweighed aluminum foil container (approximate weight, 5 mg) and dried to constant weight at 100°C for 24 hours. The weight of dry solids was obtained, and the contents were dissolved in deionized water. Sodium content was measured by flame photometry and expressed as millimoles per kilogram of dry solids.

All comparisons were by analysis of variance and regression analysis by the method of least squares using the Minitab statistics package of Pennsylvania State University on a PDP-11 digital computer (Hewlett-Packard, Palo Alto, CA, USA).

Results
The two normotensive groups studied were similar in measured characteristics. The separately recruited hypertensive subjects were older and heavier (Table 1). The intracellular ionized calcium levels were slightly but not significantly higher in the hypertensive patients, and the range of values was greater compared with those in both normotensive groups (\(p = 0.09\), SD was ± 31 for normal subjects and ± 48 for hypertensive subjects; Figure 1). There was no difference between normotensive subjects with a family history of hypertension and normal controls. In the group as a whole, there was no correlation between [Ca\(^{2+}\)], and supine blood pressure (\(r = 0.06\) for systolic; \(r = 0.24\) for diastolic; mean \(r = 0.19\)). In the normal controls (no family history), however, [Ca\(^{2+}\)], was positively correlated with supine blood pressure (\(r = +0.21\) for systolic; \(r = +0.67\) for diastolic; mean \(r = +0.73\), slope \(+2.72 \pm 0.13\) nmol/L/mm Hg mean blood pressure, \(p < 0.01\)), whereas there was no correlation in the normotensive subjects with a family history of hypertension (\(r = +0.03\) for systolic; \(r = -0.08\) for diastolic; mean \(r = +0.08\) or in the hypertensive patients, in whom there was a nonsignificant negative correlation between [Ca\(^{2+}\)], and blood pressure (\(r = -0.36\) for systolic; \(r = -0.20\) for diastolic; mean \(r = -0.31\), \(p > 0.10\); see Figure 1). There was no correlation between [Ca\(^{2+}\)], and age (\(r = -0.13\), \(p > 0.3\)) in the normal (no family history) group, nor
TABLE 1. Characteristics of the Groups Studied

<table>
<thead>
<tr>
<th>Variable</th>
<th>Normotension</th>
<th>Essential Hypertension</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No FHH</td>
<td>FHH</td>
</tr>
<tr>
<td>No. of subjects</td>
<td>21</td>
<td>17</td>
</tr>
<tr>
<td>Sex (M/F)</td>
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<td>8:9</td>
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<tr>
<td>Age (yr)</td>
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<td>Weight (kg)</td>
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<td>68 ± 3</td>
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<td>Supine BP (mm Hg)</td>
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<tr>
<td>Systolic</td>
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<td>128 ± 2</td>
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<td>Mean</td>
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<td>90 ± 2</td>
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<tr>
<td>Diastolic</td>
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<td>71 ± 3</td>
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<tr>
<td>Standing BP (mm Hg)</td>
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<tr>
<td>Systolic</td>
<td>118 ± 4</td>
<td>121 ± 3</td>
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<tr>
<td>Mean</td>
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<td>92 ± 2</td>
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<tr>
<td>Diastolic</td>
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<td>78 ± 3</td>
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<tr>
<td>[Na], (mmol/kg)</td>
<td>54 ± 3f</td>
<td>59 ± 5</td>
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<tr>
<td>[Ca2+], (nmol/L)</td>
<td>126 ± 7</td>
<td>126 ± 7</td>
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</tbody>
</table>

Data are means ± SEM. FHH = family history of hypertension; BP = blood pressure.

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

There was no difference between men and women in any group.

The white blood cell [Na], was slightly but not significantly higher in the hypertensive patients and normotensive subjects with a family history of hypertension compared with normal controls (no family history) (60 ± 7, 59 ± 5, and 54 ± 3 mmol/kg dry solids, respectively; p > 0.1). There was no correlation between [Ca2+], and [Na], in any of the groups (r < 0.07 for all groups; Figure 2).

DISCUSSION

The present study was designed to assess the possible relationship between intracellular ionized calcium in the leukocyte and blood pressure in three different groups of subjects: normotensive subjects with and without a family history of hypertension and patients with essential hypertension. The leukocyte has been used as an available, nucleated cell that might reflect a global disturbance of calcium handling in hypertensive subjects, and it has previously been reported to show abnormalities in sodium efflux in hypertensive patients and normotensive subjects with a family history of hypertension.16-18 Both the leukocyte and smooth muscle cell have adenosine 5'-triphosphate-dependent calcium efflux processes.21 The leukocyte has also been reported to have voltage-dependent calcium channels22 and to exhibit calcium-dependent processes such as chemotaxis.23-25

We were unable to demonstrate any significant elevation of [Ca2+], in leukocytes of untreated essential hypertensive patients compared with those of normotensive subjects with or without a family history of hypertension, although the mean value in this group was higher and the range of values greater, as pre-

![Figure 1. Relationship between leukocyte intracellular Ca2+ concentration ([Ca2+]i) and mean blood pressure in normotensive subjects with (○; r = +0.08) and without (●; r = +0.73) a family history of hypertension and in patients with essential hypertension (▲; r = −0.31).](http://hyper.ahajournals.org/)

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Figure 2. Relationship of leukocyte intracellular Ca\(^{2+}\) concentration ([Ca\(^{2+}\)]) to sodium content in normotensive subjects with (o) and without (*) a family history of hypertension and in patients with essential hypertension (A).

Previously reported.\(^{15}\) Hypertensive subjects were not matched for age, but there is no evidence of an age-dependent influence on leukocyte [Ca\(^{2+}\)], in this or other studies,\(^{13}\) although an age-related change in [Ca\(^{2+}\)], has been reported in rats.\(^{20}\) This finding contrasts with reports of elevated [Ca\(^{2+}\)], in platelets in hypertension\(^{13,14}\) but is in agreement with previous studies in mononuclear leukocytes and lymphocytes.\(^{14,15,27}\)

In the present study, normotensive subjects with no family history of hypertension showed a highly significant positive correlation between [Ca\(^{2+}\)], and blood pressure. This relationship is particularly impressive as the range of blood pressures was small. In view of the known role of intracellular calcium in smooth muscle contraction,\(^{1,2}\) one possible explanation for this relationship is that parallel changes occur in [Ca\(^{2+}\)], in the leukocyte and vascular smooth muscle cells in normal subjects.

To our knowledge, the failure to observe a similar relationship between blood pressure and leukocyte [Ca\(^{2+}\)], in normotensive subjects with a family history of hypertension has not been reported previously. In fact, in those studies using white blood cells, family history of hypertension is not mentioned in regard to the normotensive subjects.\(^{14,15,27}\) In the study by Erne et al.\(^{13}\) using platelets, normotensive subjects with a family history of hypertension were excluded. We found a nonsignificant negative relationship between [Ca\(^{2+}\)], and blood pressure in untreated hypertensive patients. This finding may indicate that in these two groups of subjects the relationship between [Ca\(^{2+}\)], and blood pressure is disturbed or that [Ca\(^{2+}\)], in the leukocyte and vascular smooth muscle cell no longer correlates, perhaps as the result of a disturbance in calcium handling in vascular smooth muscle that is absent in the leukocyte. Alternatively, it is possible that factors that influence blood pressure in subjects with a genetic predisposition to hypertension do not exert their effect through [Ca\(^{2+}\)]. In hypertensive patients, for instance, resistance vessel hypertrophy exerts an important effect on vascular responsiveness,\(^{28}\) although this is less likely to be important in normotensive subjects with a family history of hypertension. These subjects, however, do demonstrate an enhanced pressor responsiveness and slightly higher blood pressures compared with subjects without a family history of hypertension,\(^{28-31}\) and functional abnormalities suggestive of vascular hypertrophy have been described.\(^{32}\) Until smooth muscle [Ca\(^{2+}\)], can be adequately studied in essential hypertension, such explanations must remain speculative.

The failure to find a correlation between blood pressure and leukocyte [Ca\(^{2+}\)], in hypertensive patients contrasts with previous reports on platelet [Ca\(^{2+}\)].\(^{13}\) This could be due to differences in calcium handling by the two cell types. For example, platelets contain calcium-dependent contractile proteins and have functioning \(\alpha\)-adrenergic receptors.\(^{33,34}\) On the other hand, alterations in platelet [Ca\(^{2+}\)], may be due to disturbances independently associated with hypertension. A number of abnormalities in platelet activation and function have been reported in hypertensive patients.\(^{35-39}\)

Both white blood cells and erythrocytes from hypertensive patients show disturbances in cation fluxes. These involve several distinct pathways and differ in different populations.\(^{8,9}\) It has been suggested that increased [Na], produced as a result of such disturbances may lead to increased [Ca\(^{2+}\)], through inhibition of a postulated Na\(^+\)-Ca\(^{2+}\) exchange mechanism.\(^{40}\) Our findings do not show any relationship between [Na], and [Ca\(^{2+}\)], in the leukocyte in any of the groups, which suggests that such coupling does not occur in this tissue. This conclusion is consistent with a recently published observation that ouabain does not influence [Ca\(^{2+}\)], in mononuclear leukocytes, although no measurements of [Na], were performed in that study.\(^{15}\) There are reports that quin 2 inhibits [Na],-dependent calcium influx and therefore could have influenced these results.\(^{40}\) On the other hand, other evidence suggests that the existence of a physiologically significant
Na\(^+\)-Ca\(^{2+}\) exchange in vascular smooth muscle is doubtful.\(^{41}\)

In conclusion, the present work supports previous studies using white blood cells in failing to demonstrate a significant elevation of [Ca\(^{2+}\)], in essential hypertension, in contrast to the reports of increased levels in the platelet. However, in normotensive subjects with no family history of hypertension there was, as reported in the platelet, a relationship between [Ca\(^{2+}\)], and blood pressure. This relationship was not seen in normotensive subjects with a family history of hypertension or in patients with essential hypertension. Thus, leukocyte calcium handling may reflect a disturbance in vascular smooth muscle function in these subjects. The discrepancies in the results between platelets and white blood cells and the lack of a direct comparison between human blood cells and vascular smooth muscle in regard to sodium and calcium handling emphasize the need for caution before extrapolating these results from one tissue to the other.

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