Parameters of Lymphocyte Na\(^+\)-Ca\(^{2+}\) Regulation and Blood Pressure

The Gender Effect

Makoto Horiguchi, Masayuki Kimura, Joan Skurnick, Abraham Aviv

Abstract—Alterations in cellular Ca\(^{2+}\) and Na\(^+\) regulation play a role in the pathogenesis of essential hypertension. Using peripheral lymphocytes from 68 normal persons, we observed the following relationships for major cellular Ca\(^{2+}\) regulatory parameters. Among men and women, Na\(^+\)-Ca\(^{2+}\) exchanger activity was positively correlated with the resting cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)\(_c\)]) (\(r=0.43, P=0.0003\)), and the resting [Ca\(^{2+}\)]\(_c\) was positively correlated with cytosolic Na\(^+\) ([Na\(^+\)]\(_c\)) (\(r=0.50, P=0.0001\)). For men only, store-operated Ca\(^{2+}\) entry was positively correlated with Na\(^+\)-Ca\(^{2+}\) exchanger activity (\(r=0.63, P=0.0001\)). In addition, systolic and diastolic blood pressures were positively correlated with [Na\(^+\)], in men (\(r=0.53, P=0.001\), and \(r=0.41, P=0.017\), respectively) but not in women (\(r=0.30, P=0.088\), and \(r=0.24, P=0.17\), respectively). Some of the relationships between cellular and blood pressure parameters were confounded by serum triglycerides. These observations indicate a gender effect on cellular Ca\(^{2+}\)-Na\(^+\) regulation and its relationship with blood pressure. (Hypertension. 1998;32:869-874.)

Key Words: hypertension, essential ■ sodium ■ calcium ■ gender ■ blacks ■ ethnicity

Cytosolic Na\(^+\) ([Na\(^+\)]\(_c\)) plays a central role in controlling the cellular Ca\(^{2+}\) load in a number of cell types. The reason for this is that in these cells Na\(^+\)-Ca\(^{2+}\) exchanger (NCE) is a major transport pathway responsible for the extrusion of cellular Ca\(^{2+}\) against a steep electrochemical gradient (reviewed in Reference 1). A number of intracellular and extracellular factors have been found to modify the protein expression and activity of the cardiac NCE,\(^2,3\) the most common exchanger among the 3 known NCEs (ie, cardiac, retinal, and brain NCEs).\(^4\) However, in the final analysis, [Na\(^+\)], is a pivotal determinant of NCE activity. The relationship between [Na\(^+\)]\(_c\) and cytosolic free Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_c\)) is hence important for understanding altered regulation of cellular Ca\(^{2+}\) in essential hypertension, particularly because a major hypothesis proposes that in essential hypertension, reduced Na\(^+\)-pump activity results in a rise in [Na\(^+\)]\(_c\), inhibition of the NCE, and an increase in the cellular Ca\(^{2+}\) load.\(^5,6\) To explore this hypothesis, many studies examined the levels of [Ca\(^{2+}\)]\(_c\), and [Na\(^+\)]\(_c\) in circulating cells from humans. Platelets have been the focus of most investigations examining the relationships between [Ca\(^{2+}\)]\(_c\) and blood pressure (eg, References 7 to 13), whereas erythrocytes and to a lesser extent lymphocytes have been the main targets of studying the relationship between blood pressure and [Na\(^+\)]\(_c\) (reviewed in References 14 to 16). In a number of studies lymphocytes were also used to study [Ca\(^{2+}\)]\(_c\) regulation in relation to blood pressure.\(^15-20\)

Most of the studies in erythrocytes and lymphocytes have used flame photometry to assess cellular Na\(^+\) content and estimate [Na\(^+\)]. However, this measurement is fraught with multiple inaccuracies, including the need to estimate the intracellular and extracellular volumes to calculate the [Na\(^+\)]. (reviewed in Reference 14).

Recent investigations have shed a new light on mechanisms of external Ca\(^{2+}\) entry through store-operated Ca\(^{2+}\) channels\(^21\) and Ca\(^{2+}\) extrusion through the NCE\(^22-25\) in a number of cell types, including circulating lymphocytes. In the present investigation we used some of this new information to explore in lymphocytes the relationship between [Na\(^+\)]\(_c\) and [Ca\(^{2+}\)]\(_c\), and the relationship between major and opposing Ca\(^{2+}\) regulatory systems, namely, the NCE and store-operated Ca\(^{2+}\) entry (SOCE). To this end we used the fluorescent probes sodium-binding benzofuran isophthalate (SBFI)\(^26\) to monitor [Na\(^+\)]\(_c\), and fura 2 to monitor [Ca\(^{2+}\)]\(_c\).

Methods

Subjects

We studied 68 whites and blacks (equally divided by race and gender). They were apparently healthy and on no medications (including analgesics, oral contraceptives, and hormonal supplements). None of the blacks had sickle cell trait. After an overnight fast (that is, no food and liquids other than water after dinner the night before the study), each subject arrived at the facilities of the Hypertension Research Center between 8 and 9 AM. Heights and weights were recorded, and the subjects rested in a sitting position for a period of 15 to 20 minutes, during which information was
obtained regarding health status and family history of essential hypertension and non–insulin-dependent diabetes mellitus. Blood pressure measurements were then taken with a mercury sphygmomanometer with the subjects in a sitting position. The average of 3 blood pressure measurements, taken at 2-minute intervals, was used. Diastolic blood pressure was determined as the fifth Korotkoff sound. After blood pressure measurements were recorded, 50 mL venous blood was collected into acid dextrose buffer (20:1, vol/vol) obtained for blood chemistries. All subjects signed informed consent for blood chemistries. All subjects signed informed consents approved by the institutional review board.

**Lymphocyte Preparation**
Peripheral lymphocytes were isolated by density-gradient centrifugation as described. Cells were suspended in Ca²⁺-free HEPES buffer solution (HBS) comprising (in mmol/L): NaCl 140, KCl 5, MgCl₂ 1, glucose 10, HEPES 10, EGTA 0.3, and 0.1% BSA.

**Monitoring of [Ca²⁺] and [Na⁺]**
Lymphocytes were incubated at 37°C in either Ca²⁺-free HBS or Ca²⁺-containing HBS (ionized Ca²⁺ adjusted to 1 mmol/L). For fura 2 loading, cells were incubated for 30 minutes with fura 2-AM (5 μmol/L) (Molecular Probes, Inc), centrifuged for 5 seconds to remove external fura 2, and resuspended in Ca²⁺-free or 1 mmol/L Ca²⁺ HBS. For SBFI loading, cells were incubated with 10 μmol/L SBFI-AM (Molecular Probes) for 60 minutes, centrifuged (5 seconds) to remove the extracellular dye, and resuspended in 1 mmol/L Ca²⁺ HBS. Fluorescence of fura 2–loaded cells was monitored at excitation 340/380-nm and emission 505-nm wavelengths. Calibration of [Ca²⁺] was accomplished by exposing cells to 100 μmol/L digitonin (R_max) followed by adding 15 mmol/L EGTA (pH 8.5; adjusted to 1 mmol/L).

**TABLE 1. Subject Characteristics**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>White Men (n=16)</th>
<th>Black Men (n=18)</th>
<th>White Women (n=18)</th>
<th>Black Women (n=16)</th>
<th>P (ANOVA)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>31.4±2.33</td>
<td>31.8±1.65</td>
<td>41.1±3.38</td>
<td>40.0±2.92</td>
<td>Gender 0.001</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>115±2.14</td>
<td>114±2.73</td>
<td>109±2.80</td>
<td>110±3.12</td>
<td>NS</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>75.8±2.21</td>
<td>78.8±2.02</td>
<td>74.9±1.39</td>
<td>73.3±1.49</td>
<td>NS</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>24.8±0.50</td>
<td>26.7±0.98</td>
<td>24.2±0.89</td>
<td>26.9±0.69</td>
<td>Race 0.07</td>
</tr>
<tr>
<td>LDL, mmol/L</td>
<td>3.18±0.215</td>
<td>3.44±0.258</td>
<td>3.39±0.204</td>
<td>3.15±0.310</td>
<td>NS</td>
</tr>
<tr>
<td>HDL, mmol/L</td>
<td>1.35±0.056</td>
<td>1.18±0.051</td>
<td>1.53±0.069</td>
<td>1.32±0.069</td>
<td>Gender 0.008</td>
</tr>
<tr>
<td>Triglycerides, mmol/L</td>
<td>1.02±0.114</td>
<td>1.27±0.159</td>
<td>0.842±0.089</td>
<td>0.859±0.115</td>
<td>Gender 0.017</td>
</tr>
<tr>
<td>Glucose, mmol/L</td>
<td>5.49±0.062</td>
<td>5.46±0.156</td>
<td>5.17±0.082</td>
<td>5.36±0.091</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine, μmol/L</td>
<td>96.4±3.10</td>
<td>101±2.74</td>
<td>78.7±2.65</td>
<td>80.5±2.65</td>
<td>Gender 0.001</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; and BMI, body mass index.

**TABLE 2. Correlations Between Triglycerides, [Na⁺], and [Ca²⁺], Parameters, and Blood Pressure**

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Overall</th>
<th>Men</th>
<th>Women</th>
</tr>
</thead>
<tbody>
<tr>
<td>NCE, [Ca²⁺]ₜ</td>
<td>0.43 (0.21, 0.60)</td>
<td>0.41 (0.09, 0.66)</td>
<td>0.44 (0.12, 0.68)</td>
</tr>
<tr>
<td>P=0.0003</td>
<td>P=0.015</td>
<td>P=0.009</td>
<td></td>
</tr>
<tr>
<td>[Ca²⁺]ₜ, [Na⁺]</td>
<td>0.50 (0.30, 0.66)</td>
<td>0.46 (0.14, 0.69)</td>
<td>0.57 (0.28, 0.76)</td>
</tr>
<tr>
<td>P=0.0001</td>
<td>P=0.007</td>
<td>P=0.0005</td>
<td></td>
</tr>
<tr>
<td>SOCE, NCE</td>
<td>0.32 (0.09, 0.52)</td>
<td>0.63 (0.36, 0.80)</td>
<td>0.10 (0.25, 0.42)*</td>
</tr>
<tr>
<td>P=0.008</td>
<td>P=0.0001</td>
<td>P=0.58</td>
<td></td>
</tr>
<tr>
<td>SBP, [Na⁺]</td>
<td>0.38 (0.16, 0.57)</td>
<td>0.53 (0.23, 0.73)</td>
<td>0.30 (0.05, 0.58)</td>
</tr>
<tr>
<td>P=0.001</td>
<td>P=0.001</td>
<td>P=0.088</td>
<td></td>
</tr>
<tr>
<td>DBP, [Na⁺]</td>
<td>0.31 (0.08, 0.51)</td>
<td>0.41 (0.08, 0.65)</td>
<td>0.24 (0.11, 0.54)</td>
</tr>
<tr>
<td>P=0.010</td>
<td>P=0.017</td>
<td>P=0.17</td>
<td></td>
</tr>
<tr>
<td>SBP, [Ca²⁺]</td>
<td>0.23 (−0.01, 0.45)</td>
<td>0.19 (−0.16, 0.50)</td>
<td>0.27 (−0.08, 0.56)</td>
</tr>
<tr>
<td>P=0.057</td>
<td>P=0.28</td>
<td>P=0.12</td>
<td></td>
</tr>
<tr>
<td>DBP, [Ca²⁺]</td>
<td>0.12 (−0.12, 0.35)</td>
<td>0.004 (−0.33, 0.34)</td>
<td>0.31 (−0.03, 0.59)</td>
</tr>
<tr>
<td>P=0.32</td>
<td>P=0.98</td>
<td>P=0.073</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, [Na⁺]</td>
<td>0.39 (0.17, 0.58)</td>
<td>0.36 (0.02, 0.62)</td>
<td>0.53 (0.23, 0.74)</td>
</tr>
<tr>
<td>P=0.0009</td>
<td>P=0.039</td>
<td>P=0.001</td>
<td></td>
</tr>
<tr>
<td>Triglycerides, [Ca²⁺]</td>
<td>0.40 (0.18, 0.59)</td>
<td>0.29 (−0.05, 0.57)</td>
<td>0.61 (0.34, 0.78)</td>
</tr>
<tr>
<td>P=0.0006</td>
<td>P=0.093</td>
<td>P=0.0001</td>
<td></td>
</tr>
</tbody>
</table>
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Fluorescence in SBFI-loaded cells was monitored at excitation 340/385-nm and emission 505-nm wavelengths. Autofluorescence was taken with the use of dye-unloaded cells. Calibration of [Na\(^{+}\)] was performed with modification of a previously described protocol. Briefly, gramicidin D (2 \(\mu\)mol/L), nigericin (5 \(\mu\)mol/L), and monensin (5 \(\mu\)mol/L) were added to calibration solutions comprising (in mmol/L): Na gluconate 0 to 115, K gluconate 0 to 115 (where Na gluconate+K gluconate=115), KCl 30, CaCl\(_2\) 1, MgCl\(_2\) 1, glucose 10, and HEPES 10 (pH 7.4). Eight concentrations of Na\(^{+}\) were used in the calibration solutions, ie, 0, 5, 10, 20, 30, 50, 80, and 115 mmol/L. Fluorescence was monitored in stirred cells at 37°C in a Fluorolog II spectrofluorometer (SPEX Industries).

**Na\(^{+}\)-Ca\(^{2+}\) Exchanger Activity**

Na\(^{+}\)-dependent external Ca\(^{2+}\) entry was taken as maximal NCE activity in the reverse mode. The following steps were undertaken to measure this parameter: Ouabain (0.1 mmol/L) was added during the last 10 minutes of incubation with fura-2 AM in 1 mmol/L Ca\(^{2+}\)-containing HBS. Cells were rapidly centrifuged and resuspended in either (1) Li\(^{+}\)-containing, Ca\(^{2+}\)-free HBS, comprising (in mmol/L): LiCl 140, KCl 10, MgCl\(_2\) 1, glucose 10, HEPES 10, and 0.1% BSA, or (2) Na\(^{+}\)-containing, Ca\(^{2+}\)-free HBS, in which LiCl was equimolarly replaced by NaCl. One millimole per liter (final concentration) of ionized Ca\(^{2+}\) plus 1 \(\mu\)mol/L thapsigargin were added, and the Ca\(^{2+}\) signal was monitored for 45 seconds. The difference in the [Ca\(^{2+}\)]

**Store-Operated Ca\(^{2+}\) Entry**

Thapsigargin (1 \(\mu\)mol/L) was added during the last 10 minutes of incubation with fura-2 AM in Ca\(^{2+}\)-free HBS. Cells were rapidly centrifuged and resuspended in Ca\(^{2+}\)-free HBS. Ca\(^{2+}\) (0.5 mmol/L final concentration) was rapidly added to the medium, and the Ca\(^{2+}\) signal was monitored for 45 seconds.

**Other Measurements**

Levels of total serum cholesterol, HDL cholesterol, triglycerides, glucose, and creatinine were measured in a Kodak Ectachem DT 60 analyzer.

**Data Analysis**

The relationships among sodium and calcium parameters and physiological measurements were analyzed by simple linear correlation and regression, partial correlation, and multiple regression. The effects of gender and race were analyzed by ANOVA. All calculations were performed with SAS statistical software (SAS Institute, Inc). The criterion for statistical significance was \(P<0.05\).

**Results**

Table 1 presents the general characteristics of the subjects: women were older than men, and the body mass index was greater in blacks than in whites. HDL levels were higher in women than in men and in whites than in blacks. In addition, serum creatinine was higher in men than in women, reflecting the greater muscle mass in men.

Table 2 shows correlations among parameters for all subjects combined and separately for men and women. No differences in correlations were observed between blacks and whites. NCE activity was strongly correlated with the resting [Ca\(^{2+}\)]

**Figure 1. Relationships between [Na\(^{+}\)]

and [Ca\(^{2+}\)]

in lymphocytes from men (A) and women (B). Closed symbols indicate blacks; open symbols, whites.

**Figure 2. Relationship between NCE activity and SOCE in men.** Closed symbols indicate blacks; open symbols, whites.
with $[Na^+]$ and with NCE activity remained strong (partial correlation coefficients $r=0.41$, $P=0.0006$, and $r=0.54$, $P=0.0001$, respectively), as did the correlation of SOCE and NCE activity (partial correlation coefficient $r=0.31$, $P=0.013$). Correlations of $[Na^+]$, with systolic and diastolic blood pressures were attenuated by adjustment for triglycerides (partial correlation coefficients $r=0.23$, $P=0.058$, and $r=0.20$, $P=0.11$, respectively). After adjustment for triglycerides, the relationship between systolic blood pressure and resting $[Na^+]$ did not differ between men and women.

No associations were observed between lymphocyte parameters and familial history of essential hypertension or non–insulin-dependent diabetes.

**Discussion**

A relevant finding of this work is the strong positive correlation between the resting $[Ca^{2+}]$, and $[Na^+]$, in lymphocytes from a heterogeneous human population. This was observed in both men and women. Such a finding strongly suggests that $[Na^+]$, and therefore the NCE play important roles in the regulation of the resting $[Ca^{2+}]$, in human lymphocytes. Other related findings of interest are observations that the resting $[Ca^{2+}]$, was strongly correlated with NCE in both men and women, suggesting that as $[Ca^{2+}]$ rises, adaptive mechanisms are initiated to enhance $Ca^{2+}$ extrusion through the NCE. This is probably mediated by the upregulation of NCE expression, since in the present investigation we examined the activity of NCE (in a reverse mode) at high (1 mmol/L) $Ca^{2+}$ concentration, reflecting the maximal reaction velocity of the exchanger. Such a concept awaits proof by direct quantification of NCE protein.

Until recently it has been difficult to show the presence of NCE in human lymphocytes.27 Perhaps one reason for this difficulty relates to the substantial capacity of the sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase to sequester $Ca^{2+}$ and thus confluence in the sarco(endo)plasmic reticulum with little effect on the level of the resting $[Ca^{2+}]$. However, in human lymphocytes $45Ca^{2+}$ fluxes, and $[Ca^{2+}]$, after inhibition of the sarco(endo)plasmic reticulum $Ca^{2+}$-ATPase, are greatly modified by removal of external $Na^+$ and by treatment with ouabain (reviewed in Reference 23). These findings support the presence of NCE in these cells.

Oshima et al20,29 have demonstrated a strong correlation between $[Na^+]$, (measured by flame photometry) and $[Ca^{2+}]$, in lymphocytes from hypertensive and normotensive subjects. Although these investigators showed higher levels of lymphocyte $[Ca^{2+}]$, and $[Na^+]$, in patients with essential hypertension than in normotensive controls,19,20,29 they did not find correlations for the levels of these ions with blood pressure when blood pressure was evaluated as a continuously distributed variable.19,20,29 The present study and our previous work17 have confirmed the lack of correlation between the resting $[Ca^{2+}]$, in lymphocytes and blood pressure. However, we did show a strong correlation between the $[Na^+]$, in lymphocytes and blood pressure.

**TABLE 3. Partial Correlation Coefficients, Adjusted for Triglycerides**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Partial Correlation Coefficient (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>$[Ca^{2+}]$, $[Na^+]$</td>
<td>0.41 (0.19, 0.59)</td>
<td>0.0006</td>
</tr>
<tr>
<td>SOCE, NCE</td>
<td>0.31 (0.07, 0.51)</td>
<td>0.013</td>
</tr>
<tr>
<td>$[Ca^{2+}]$, NCE</td>
<td>0.54 (0.34, 0.69)</td>
<td>0.0001</td>
</tr>
<tr>
<td>SBP, $[Na^+]$</td>
<td>0.23 (−0.07, 0.45)</td>
<td>0.058</td>
</tr>
<tr>
<td>DBP, $[Na^+]$</td>
<td>0.20 (−0.05, 0.42)</td>
<td>0.11</td>
</tr>
</tbody>
</table>

SBP indicates systolic blood pressure; DBP, diastolic blood pressure.
and blood pressure in men but not in women. One possible explanation is that in the present work the use of SBFI facilitated a more sensitive measurement of \([\text{Na}^+]_c\), and uncovered the relationship between the cellular parameter and blood pressure. It is possible that variation in \([\text{Na}^+]_c\) (a parameter not evaluated in this work) accounts for some variation in lymphocyte \([\text{Na}^+]\) and \([\text{Ca}^{2+}]_c\) regulation. Nonetheless, the relationship among the cellular parameters and between lymphocyte \([\text{Na}^+]\) and blood pressure should hold irrespective of variation in \([\text{Na}^+]_c\) intake.

It is noteworthy that gender, presumably through the effects of ovarian steroid hormones, plays a role in modifying the relationship between blood pressure and a number of molecular, cellular, and systemic modalities, including polymorphism in the angiotensinogen locus,\(^{30}\) and the activities of \(\text{Na}^-\text{Li}^+\) exchange in erythrocytes\(^{31,32}\) and \([\text{Na}^+]_c\) and \([\text{Ca}^{2+}]_c\) exchange in platelets.\(^{33}\) In addition, gender modifies the expression of non-modulation\(^{34}\) and urinary excretion of cortisol.\(^{35}\) These phenotypes reflect variables involved in \([\text{Na}^+]\) regulation at the cellular and systemic levels. Their dependency on gender is therefore in accordance with our findings that the relationship between lymphocyte \([\text{Na}^+]\), and blood pressure is stronger for men than for women. The gender-dependent effect was shown in our investigations not only for the relationship between SOCE and the NCE but perhaps also for the relationship between \([\text{Na}^+]\), and blood pressure.

The positive correlation between \([\text{Ca}^{2+}]_c\) and \([\text{Na}^+]\), in lymphocytes suggests that, through its effect on the NCE, \([\text{Na}^+]\), modifies \([\text{Ca}^{2+}]_c\). It is noteworthy that the resting \([\text{Ca}^{2+}]_c\), might poorly reflect the overall cellular \([\text{Ca}^{2+}]_c\) load, since the bulk of the freely exchangeable \([\text{Ca}^{2+}]_c\) is sequestered within the sarco(endo)plasmatic reticulum. A number of investigations have demonstrated that in platelets, the resting \([\text{Ca}^{2+}]_c\), correlates with blood pressure.\(^{7,9,12}\) Other studies, including a number of studies by our group, have found no statistical correlations between the resting \([\text{Ca}^{2+}]_c\), in platelets and blood pressure when the blood pressure was evaluated as a continuously distributed variable.\(^{10,25,36}\) In our more recent work in platelets, we found that although the resting \([\text{Ca}^{2+}]_c\), was not correlated with blood pressure, the freely exchangeable \([\text{Ca}^{2+}]_c\) in the sarco(endo)plasmatic reticulum (dense tubules) of platelets was strongly correlated with blood pressure, but only in men\(^{25}\) and not in women.\(^{37}\) These observations further underscore the role of gender in shaping cellular \([\text{Ca}^{2+}]_c\) and \([\text{Na}^+]\) regulation.

We previously reported,\(^{25}\) as in the present work, that serum triglycerides correlated with \([\text{Ca}^{2+}]_c\) parameters in circulating cells. This conclusion is not confined to our studies on the relationship between cellular ion regulation and blood pressure. For instance, other groups have repeatedly shown that serum triglycerides correlated with the activity of the \([\text{Na}^-\text{Li}^+]_c\) exchange in erythrocytes and therefore modified the relationship between this transport system and blood pressure.\(^{38–42}\) Our findings are relevant in that they clearly show that triglycerides do not modify the interrelationships between the various cellular \([\text{Ca}^{2+}]_c\) (and \([\text{Na}^+]_c\)) parameters but the relationships between these parameters and blood pressure. In this regard, the variation in lymphocyte parameters might not be causally related to variation in triglycerides. Rather, both might be pleiotropic expressions of variant genes.

In conclusion, we report here that \([\text{Na}^+]\), in peripheral lymphocytes of a heterogeneous human population positively correlates with resting \([\text{Ca}^{2+}]_c\), and that \([\text{Na}^+]\), strongly correlates with blood pressure, particularly in men. Fluctuating levels of ovarian steroid hormones in premenopausal women might account for the sexual dimorphism in the expression of hypertension\(^{43}\) and for the effect of gender on the relationship among cellular variables of \([\text{Ca}^{2+}]_c\) and \([\text{Na}^+]_c\) regulation and on the relationship between \([\text{Na}^+]\), and blood pressure in humans.

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