Relation of Cellular Potassium to Other Mineral Ions in Hypertension and Diabetes

Lawrence M. Resnick, Mario Barbagallo, Ligia J. Dominguez, Joseph M. Veniero, J.P. Nicholson, Raj K. Gupta

Abstract—To investigate the role of intracellular potassium (K) and other ions in hypertension and diabetes, we utilized \(^{39}\text{K}, {^{23}\text{Na}}, {^{31}\text{P}}\), and \(^{19}\text{F}\)-nuclear magnetic resonance (NMR) spectroscopy to measure K, intracellular sodium (Na), intracellular free magnesium (Mg), and cytosolic free calcium (Ca), respectively, in red blood cells of fasting normotensive nondiabetic control subjects (n=10), untreated (n=13) and treated (n=14) essential hypertensive subjects, and diabetic subjects (n=5). In 12 subjects (6 hypertensive and 6 normotensive controls), ions were also measured before and after the acute infusion of 1 L of normal saline. Compared with those in controls (K=148±2.0 mmol/L), K levels were significantly lower in hypertensive (132.2±2.9 mmol/L, sig=0.05) and in type 2 diabetic subjects (121.2±6.8 mmol/L, sig=0.05). K was higher in treated hypertensives than in untreated hypertensives (139±3.1 mmol/L, sig=0.05) but was still lower than in normals. Although no significant relation was observed between basal K and Na values, saline infusion elevated Na (P<0.01) and reciprocally suppressed K levels (142±2.4 to 131±2.2 mmol/L, P<0.01). K was strongly and inversely related to Ca (r=-0.846, P<0.001), and was directly related to Mg (r=0.664, P<0.001). We conclude that (1) K depletion is a common feature of essential hypertension and type 2 diabetes, (2) treatment of hypertension at least partially restores K levels toward normal, and (3) fasting steady-state K levels are closely linked to Ca and Mg homeostasis. Altogether, these results emphasize the similar and coordinate nature of ionic defects in diabetes and hypertension and suggest that their interpretation requires an understanding of their interaction. (Hypertension. 2001;38[part 2]:709-712.)

Key Words: potassium ■ diabetes ■ calcium ■ magnetic resonance spectroscopy ■ hypertension

Cellular responses to a variety of stimuli depend on the maintenance of normal intracellular potassium (K) stores, which determine the state of cell membrane potential.\(^{1}\) K homeostasis is also linked to intracellular sodium (Na), calcium (Ca), and magnesium (Mg) metabolism, via Na\(^+-\)K\(^+-\)ATPase, Ca\(^{2+}\)-activated K channels, and other mechanisms.\(^{2-4}\) Thus, the pressor response to dietary salt loading, associated with increases in Na and Ca, and depletion of Mg,\(^{5}\) is blunted by increased K intake.\(^{6}\) Dietary K intake has also been linked with the cerebrovascular disease risk associated with aging and hypertension.\(^{7,8}\) The hypothesis emerging from these and other studies, that a cellular K deficiency directly contributes to hypertension-associated diseases, has been tested by measuring K or surrogate (rubidium) ion flux rates, membrane-related enzyme ion pump activities, and K, although often in broken cell preparations or in cells suspended in artificial media before analysis.\(^{1,9-12}\)

We have utilized nuclear magnetic resonance (NMR) spectroscopic techniques to noninvasively assess steady-state intracellular ion concentrations under conditions that closely represent their native environment.\(^{13-17}\) In this study, we measured K levels in erythrocytes of normal, essential hypertensive, and diabetic subjects, and compared these levels to concomitant levels of other intracellular mineral ions. Our results support the hypothesis that a cellular deficiency of K exists in essential hypertension and in type 2 diabetes mellitus. Furthermore, our data demonstrate close relationships between K, and levels of other ions measured concomitantly, thus emphasizing the coordinate nature of intracellular ion homeostasis.

Methods

Patients

All subjects were recruited at the Hypertension Center of the New York Presbyterian Hospital–Cornell Medical Center. Normotensives (NI, n=10) were recruited from an epidemiologic study of a normal population (NIH-SCOR). Unmedicated essential hypertensive subjects (HiBP, n=13) were diagnosed on the basis of 3 independent BP readings >150/95 mm Hg, of being off all medications for ≥3 weeks, and of the absence of any history, physical examination, or laboratory evidence of secondary hypertension. Medicated hyperten-
sive subjects (HiBP-Rx, n=14) with normal BP values were also studied. Medications included dihydropyridine calcium antagonists (n=7), ACE inhibitors (n=5), β-blockers (n=4), α-blockers (n=1), and diuretics (n=1). Five Ni subjects had fasting hyperglycemia (fasting blood sugar=8.7±0.6 mmol/L) on 2 separate occasions and were considered a subgroup with new-onset type 2 diabetes mellitus.

Heparinized blood was drawn in quietly seated patients who arrived between 9:00 and 10:00 AM after an overnight fast. In some subjects, additional blood was also drawn for measurement of Ca$_i$, and/or Mg$_i$ content. In a separate group of 12 fasting unmedicated HiBP and Ni subjects (6 hypertensive and 6 normotensive, 9 male/3 female) participating in a saline infusion protocol, blood for Na$_i$ and K$_i$ ion measurements was drawn before and 30 minutes after infusion of 1000 mL of 0.9% NaCl. All intracellular ion analyses were performed at the Albert Einstein College of Medicine.

**Intracellular Na$^+$**

Ten milliliters of blood was mixed with the paramagnetic shift reagent, dysprosium bis(tripolyphosphate) (Dy$[PPPi]_2$)$^{3-}$, to a concentration of 5 mmol/L and then spun at 2000 rpm for 10 minutes; the plasma and cells were put into separate 10-mm NMR tubes. $^{23}$Na-NMR spectra were obtained on a Varian XL-200 spectrometer operating at 25.9 MHz. The Na$_i$ concentration was calculated as Na$_i$ = (Na$^i$(cell)/A$_{cell}$-Na$^i$(plasma)/A$_{plasma}$)$(1-S_{extr})$, where A$_{cell}$ and A$_{plasma}$ are the areas of the intracellular and extracellular resonances, respectively, S$_{extr}$ is the fractional extracellular volume (A$_{out}$/A$_{in}$), and [Na$^{++}$]$_{in}$ is the plasma Na$^{+}$ concentration, obtained independently by standard techniques.$^{17}$

**Intracellular K$^+$**

K$^+$ spectra were obtained on the same sample of packed erythrocytes used for the Na$_i$ determination, using a $^1$H-NMR probe on a Varian VXR-500 spectrometer operating at 23.3 MHz.$^{16}$ Integration of the K$^+$ resonance and comparison of this area to that of a standard K$^+$ reference (150 mmol/L) allows for the calculation of K$^+$ as K$_i$ = (K$^+$(cell)/A$_{cell}$(K$^+$)-A$_{plasma}$(K$^+$))(1-(S$_{extr}$-1)) where A$_{cell}$ and A$_{plasma}$ are the areas of the intracellular unknown sample and standard solution $^1$H resonance, respectively, and S$_{extr}$ is the fractional extracellular volume as determined for the calculation of Na$_i$.

**Cytosolic Free Calcium**

Ten milliliters of blood was spun at 10 000 rpm for 10 minutes, and the plasma was removed and saved. The packed cells were loaded for 20 minutes at 37°C with 20 mmol/L QUIN-MF in 100 mL of Hank's balanced salt solution (HBSS) titrated with NaHCO$_3$ to a pH of 7.4, and the cells were decanted into an NMR tube.$^{19}$ F-NMR spectra were recorded at 37°C on a Varian VXR-500 spectrometer operating at 470.4 MHz. Ca$_i$ levels were calculated as Ca$_i$ = (Ca$_i$(QUIN-MF)/A$_{Ca$-QUIN-MF}$), where A$_{Ca$-QUIN-MF}$ and A$_{QUIN-MF}$ are the areas of the calcium-bound and free QUIN-MF resonances, respectively; and K$_d$ is the apparent dissociation constant of the Ca-QUIN-MF complex. This constant has been determined to be approximately 139 mmol/L in our laboratory.

**Intracellular Free Magnesium**

Heparinized blood was centrifuged, and the packed cells decanted into 10-mm NMR tubes for analysis. All spectra were obtained on a Varian XL 200 spectrometer, operating at 37°C.$^{18}$ Mg$_i$ levels were determined according to the formula: Mg$_i$ = (K$_d$(MgATP)/$^1$P - 1)), where $^1$P$_{(}$ is the free unbound fraction of ATP, is calculated from the chemical shift differences of the α- and β-phosphoryl resonances of ATP in the $^3$P-NMR spectrum, and K$_d$(MgATP) = 38 μmol/L at 37°C.

**Statistical Analysis**

K$_i$ and Na$_i$ values obtained from the different patient groups were analyzed and compared using 1-way ANOVA, with post-hoc t tests adjusted for multiple comparisons (Bonferroni). Paired t tests were used to compare K$_i$ and Na$_i$ values before and 30 minutes after the intravenous NaCl infusion. When Na$_i$, Ca$_i$, and/or Mg$_i$ were measured concomitantly, the relation between these ions and K$_i$ values were analyzed using linear regression analysis and Pearson correlation coefficients. Results are expressed as mean±SEM.

**Results**

The patient groups analyzed did not differ in average age, gender, or racial distribution; BMI; or renal function (Table). Systolic BP was significantly higher in the unmedicated essential hypertensive group but did not differ significantly among the other patient groups studied, whereas for diastolic BP a small but significantly difference was also present in the hypertensive treated patients compared with the normotensive controls (Table).

<table>
<thead>
<tr>
<th>Characteristics of Study Subjects</th>
<th>HiBP (n=14)</th>
<th>HiBP-Rx (n=15)</th>
<th>Type 2 Diabetes (n=5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>57±6</td>
<td>56±6</td>
<td>54±2</td>
</tr>
<tr>
<td>Gender, M/F</td>
<td>5/5</td>
<td>4/9</td>
<td>5/9</td>
</tr>
<tr>
<td>Race, B/W</td>
<td>2/8</td>
<td>3/10</td>
<td>3/11</td>
</tr>
<tr>
<td>BMI, kg/m$^2$</td>
<td>27.8±3.1</td>
<td>28.9±2.2</td>
<td>30.0±3.4</td>
</tr>
<tr>
<td>SBP, mm Hg</td>
<td>132±6.6</td>
<td>162±6.6</td>
<td>138±7</td>
</tr>
<tr>
<td>DBP, mm Hg</td>
<td>73±3</td>
<td>99±3.5</td>
<td>81±3.4</td>
</tr>
<tr>
<td>Cr, mg/dL</td>
<td>0.8±0.05</td>
<td>0.9±0.07</td>
<td>1.0±0.07</td>
</tr>
<tr>
<td>K$_i$, mmol/L</td>
<td>148±2.0</td>
<td>132.2±2.9</td>
<td>139±3.1†</td>
</tr>
<tr>
<td>Na$_i$, mmol/L</td>
<td>9.7±0.8</td>
<td>9.0±0.8</td>
<td>9.8±0.9</td>
</tr>
</tbody>
</table>

*P<0.05 vs Ni.
†P<0.05 vs HiBP-No Rx.
to 131±2.2 mmol/L, *P*<0.001. K<sub>i</sub> before and after NaCl infusion was quantitatively related to the concomitantly obtained Na<sub>i</sub> levels (r=0.736, *P*<0.001). These results were consistent among all the subjects studied, independently of blood pressure or gender status.

**Discussion**

Epidemiologic data suggest that dietary intakes of NaCl, K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> may all contribute to hypertension and its consequences. Conversely, hypertension is ameliorated by increasing K<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> or by decreasing NaCl intake. Presumably, these dietary alterations influence ionic events at the cellular level, leading to altered tissue function, but this has been difficult to determine because previous measurements of steady state cellular ion content of, eg, K<sup>+</sup>, often required cell suspension in artificial media, the use of chelating agents that distribute not only into cytosol but into other cell compartments as well, or even the disruption of cell membranes. Furthermore, focusing on single ions rather than on their mutual interaction often leads to artificial controversies in which K<sup>+</sup>, Na<sup>+</sup>, Ca<sup>2+</sup>, and Mg<sup>2+</sup> have each been claimed as the "most" important ionic determinant of pathologic processes such as hypertension.

Our group has developed NMR spectroscopic techniques to noninvasively and coordinately analyze steady-state cellular ion content. Although limited by the greater time course and amounts of tissue required for analysis, NMR techniques possess certain advantages, including greater precision and reproducibility and a closer preservation of the native extracellular environment in which the analyses are performed. Utilizing these NMR techniques in the present study, we found the following: (1) compared with normotensive subjects, fasting K<sub>i</sub> levels are significantly lower in red cells obtained from untreated human essential hypertensive subjects; (2) in normotensive and hypertensive subjects, BP is inversely related to K<sub>i</sub> content—the lower the K<sub>i</sub>, the higher the pressure; (3) medicated hypertensives exhibited significantly higher K<sub>i</sub> levels compared with those of untreated hypertensive subjects; and (4) untreated type 2 diabetic subjects exhibit a greater degree of K<sub>i</sub> depletion. Furthermore, (5) K<sub>i</sub> levels are closely linked to levels of other intracellular ions. Specifically, steady-state fasting Ca<sub>i</sub> and Mg<sub>i</sub> levels are, respectively, inversely and directly related to K<sub>i</sub>—the higher the K<sub>i</sub>, the lower the Ca<sub>i</sub> (Figure 1A) and the higher the Mg<sub>i</sub> (Figure 1B). Lastly, (6) although no basal relation was observed between K<sub>i</sub> and Na<sub>i</sub> levels, they were closely and inversely linked during NaCl infusion, where Na<sub>i</sub> rose and K<sub>i</sub> reciprocally fell (Figure 2). Altogether, these data document deficient K<sub>i</sub> levels in hypertension and diabetes, demonstrate the relationship between monovalent and divalent cellular cations, and emphasize the need to interpret studies of cellular ion content accordingly.

What might be the possible pathophysiologic significance of these findings? First, although our data, obtained in red blood cells, need to be confirmed in other tissues, such as vascular smooth muscle (VSM), the depletion of cellular K<sub>i</sub> or Mg<sub>i</sub>, and/or the Ca<sub>i</sub> excess can each directly produce or predispose to VSM contraction, increased constrictor tone, increased BP, insulin resistance, and abnormalities of glucose and insulin metabolism. Thus, although the causal mechanisms of these ionic changes have yet to be defined, the observation here of K<sub>i</sub> depletion in hypertension and diabetes and its strong linkage with Mg<sub>i</sub> and Ca<sub>i</sub> levels further supports the notion of increased vasoconstrictor tone and insulin resistance as different tissue manifestations of a common cellular ionic defect. Indeed, cellular depletion of K<sub>i</sub> and Mg<sub>i</sub> is associated with elevated blood lipid levels, atherosclerosis, altered endothelial function, and decreased biosynthesis and release of NO. Second, the coordinate nature of these cellular ionic lesions—ie, abnormal levels of any 1 ion reflecting linked abnormalities of others—suggests the somewhat artificial nature of controversies arising out of claims that 1 particular cellular ionic species is more important than another. It may be rather, that independently of the nature of the initial lesion, which might differ in different genetically or environmentally determined forms of hypertension and/or diabetes (and which may or may not involve...
primary ion-related events), that the alterations of cellular ion content observed here participate in a final common pathway necessary for the emergence of the insulin resistant and/or hypertensive state. At the very least, our results confirm the involvement of deficient \( K^+ \) in hypertension and diabetes, emphasize the interaction and interdependency of \( K^+ \) with \( Mg^{2+} \) and \( Ca^{2+} \), and suggest that in the future, proper interpretation of cellular events in these disease states warrants their concomitant measurement.

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References

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