Magnesium Responsiveness to Insulin and Insulin-Like Growth Factor I in Erythrocytes from Normotensive and Hypertensive Subjects

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ABSTRACT

Depletion of intracellular free magnesium (Mg) is a characteristic feature of insulin resistance in essential hypertension, but it is not clear to what extent low Mg levels contribute to insulin resistance, result from it, or both. As insulin-like growth factor I (IGF-I) may improve insulin resistance, we investigated whether this peptide could similarly improve Mg responsiveness to insulin in hypertension, and whether this effect was related to any direct IGF-I effect on Mg. In erythrocytes from 13 fasting normotensive and 10 essential hypertensive subjects before and 30, 60, and 120 min after incubation with a physiologically maximal dose of insulin (200 μU/mL) and with different doses of recombinant human IGF-I (0.1–100 nmol/L).

In normotensive subjects, IGF-I elevated Mg (P < 0.05) in a dose- and time-dependent fashion, as did insulin (P < 0.05). However, in hypertensive subjects, maximal Mg responses to insulin, but not to IGF-I, were blunted (insulin, 163 ± 11 to 177 ± 10 μmol/L [P = NS]; IGF-I, 164 ± 6 to 190 ± 11.7 μmol/L [P < 0.05]). Furthermore, for insulin, but not for IGF-I, cellular Mg responsiveness was closely and directly related to basal Mg levels (insulin: r = 0.72; P < 0.01; IGF-I: r = 0.18; P = NS). Lastly, blunted Mg responses to insulin could be reversed by preincubation of hypertensive cells with IGF-I.

We conclude that 1) both IGF-I and insulin stimulate erythrocyte Mg levels; 2) cellular Mg responses to insulin, but not to IGF-I, depend on basal Mg levels, i.e. the higher the Mg, the greater the sensitivity to insulin; and 3) IGF-I potentiates insulin-induced stimulation of Mg at doses that themselves do not raise Mg. These effects of IGF-I may underlie at least in part its ability to improve insulin sensitivity clinically. Together, these data support a role for IGF-I in cellular magnesium metabolism and emphasize the importance of magnesium as a determinant of insulin action. (J Clin Endocrinol Metab 83: 4402–4407, 1998)

THE CLINICAL and epidemiological association between syndromes characterized by insulin resistance, such as essential hypertension, obesity, and noninsulin-dependent diabetes mellitus (NIDDM), is well known (1–4). Erythrocytes and other cells from subjects with these syndromes display higher cytosolic free calcium levels (Ca), reciprocally lower cytosolic free magnesium (Mg) levels, altered intracellular pH (pH), and other ionic abnormalities (5–7). These abnormalities have, in turn, been closely related to the level of blood pressure (5), the extent of cardiac hypertrophy (8), and the degree of insulin resistance present in these clinical states (6). On the basis of these and other data, our group has proposed an ionic hypothesis in which the above cellular abnormalities explain the association of these syndromes with different clinical manifestations of a common shared ionic defect (9). According to this hypothesis, the insulin resistance of hypertension, obesity, and NIDDM results at least in part from a cellular Mg deficiency. However, it has been difficult to determine the precise role of the cellular Mg deficit in causing insulin resistance, as this deficit might also be the secondary result of resistance to the direct Mg-elevating actions of insulin (10, 11).

To help resolve this question, we have begun to investigate the cellular actions of insulin-like growth factor I (IGF-I), a circulating polypeptide with vasoactive (12, 13), growth-promoting, and metabolic properties similar to those of insulin (14, 15). IGF-I can exert its biological action both through specific IGF-I receptors and, because of sequence homology with insulin, through the insulin receptor (16). Acute and long term metabolic effects of human recombinant IGF-I have been demonstrated in vitro (17) and in vivo (18, 19), including its ability to ameliorate insulin resistance in diabetes (20, 21).

Therefore, using 31P-nuclear magnetic resonance (31P-NMR) spectroscopic techniques, we examined intracellular free magnesium responses to insulin and IGF-I in erythrocytes from normal and essential hypertensive individuals. Our results demonstrate that both IGF-I and insulin stimulate Mg; that in hypertensive subjects the action of insulin, but not that of IGF-I, is blunted; and that the cellular Mg effects of insulin appear closely linked to basal Mg levels. These data suggest that the ability of IGF-I to improve insulin sensitivity clinically may be related at least in part to its effect on cellular Mg metabolism.

Subjects and Methods

Twenty milliliters of venous blood were drawn from unmedicated normotensive (NT; n = 13) and hypertensive (HTN; n = 10) subjects in the morning (0900–1200 h) after an overnight fast. The patients included in the study were randomly selected from the outpatient hypertension
were plotted against the pH value at which the spectrum was obtained. A titration curve, prepared by analyzing spectra obtained at various known pH values, was linear within the pH range tested and was used to determine the pH of unknowns.

**Statistical analyses**

The statistical significance of differences in Mg, responses to each hormone treatment vs. basal (no treatment) was estimated by ANOVA, using the appropriate post-hoc t test for multiple comparisons. The relations between measured variables were assessed by linear regression analysis and Pearson correlation coefficients. Statistical tests were performed using the CRUNCH software package on an IBM-compatible computer. All data are presented as the mean ± SEM. P < 0.05 was considered statistically significant.

**Results**

Basal Mg, values (in micromoles per L) were lower in erythrocytes from HTN compared to erythrocytes from NT subjects (168 ± 7.8 vs. 187 ± 8.8; P < 0.05). IGF-I elevated Mg, in erythrocytes from NT and HTN subjects at a threshold dose of 10 nmol/L (Fig. 1). The effect of IGF-I was observed at 60 and 120 min of incubation (P < 0.05 at each time vs. basal; Fig. 2). By contrast, insulin-stimulated Mg, levels in cells from NT, but not HTN, subjects (Fig. 2). These different Mg, responses to IGF-I vs. insulin in HTN and NT cells were sufficient both for maximal absolute Mg, levels attained and

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**TABLE 1. Clinical and laboratory characteristics of study subjects**

<table>
<thead>
<tr>
<th></th>
<th>Normotensive (n = 10)</th>
<th>Hypertensives (n = 13)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yr)</td>
<td>44 ± 5</td>
<td>47 ± 6</td>
<td>NS</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>5/5</td>
<td>6/7</td>
<td>NS</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.8 ± 1.0</td>
<td>26.1 ± 0.9</td>
<td>NS</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>132 ± 4</td>
<td>151 ± 8</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>78 ± 3</td>
<td>98 ± 5</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>16.0 ± 0.9</td>
<td>16.9 ± 1.1</td>
<td>NS</td>
</tr>
<tr>
<td>Creatinine (mg/dL)</td>
<td>0.9 ± 0.05</td>
<td>1.0 ± 0.06</td>
<td>NS</td>
</tr>
<tr>
<td>FBG (mg/dL)</td>
<td>92 ± 4</td>
<td>96 ± 5</td>
<td>NS</td>
</tr>
<tr>
<td>CaT (mg/dL)</td>
<td>9.7 ± 0.1</td>
<td>9.7 ± 0.1</td>
<td>NS</td>
</tr>
<tr>
<td>MgT (mg/dL)</td>
<td>1.89 ± 0.03</td>
<td>1.87 ± 0.04</td>
<td>NS</td>
</tr>
</tbody>
</table>

BMI, Body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; BUN, blood urea nitrogen; FBG, fasting blood glucose; Cat, serum total calcium; MgT, serum total magnesium.

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**FIG. 1.** Dose-response curve of the effect of IGF-I on erythrocyte 31P-NMR-determined Mg, levels in NT (A) and HTN (B) individuals. Each point represents the mean ± SEM of 13 and 10 subjects, respectively. *P < 0.05; **P < 0.01 (vs. basal Mg, levels).
for the change at 30, 60, and 120 min in Mg_i (in micromoles per L) from baseline values (ΔMg_i for insulin: in NT, 28.9 ± 5.0, 30.0 ± 8.0, and 28.3 ± 8.0; in HTN, 0.2 ± 3.1, 2.8 ± 3.4, and −2.9 ± 3.0; ΔMg_i for IGF-I: in NT, 7.1 ± 5.1, 25.5 ± 5.2, and 41.9 ± 5.6; in HTN, 2.0 ± 2.0, 18.8 ± 6.8, and 20.6 ± 7.5). No significant effects of IGF-I or insulin on pH_i levels were detected in this in vitro erythrocyte system at any of the times examined (Table 2).

For all subjects, regardless of diagnostic clinical blood pressure category, the cellular Mg_i responsiveness to insulin was closely linked to basal Mg_i (r = 0.72; P < 0.01); the lower the basal Mg_i level, the more blunted the cell Mg_i response (Fig. 3A). This was not true for IGF-I, where stimulation of Mg_i occurred independently of basal Mg_i levels (r = 0.18; P = NS; Fig. 3B).

Lastly, using doses of insulin (200 μU/mL) and IGF-I (1 nmol/L) that did not themselves alter Mg_i levels individually, a significant rise in Mg_i was observed when insulin was added to IGF-I-preincubated cells (Fig. 4).

**Discussion**

In this investigation we compared insulin’s ionic effects with those of IGF-I, whose effect on Mg_i levels has not been previously studied. We observed 1) that insulin at physiologically maximal concentrations, which significantly elevate Mg_i levels in cells from NT subjects, failed to elevate Mg_i in cells from HTN subjects; 2) that for all subjects, independently of their designation as NT or HTN, Mg_i responsiveness to insulin was closely and directly related to basal Mg_i levels, i.e. the lower the basal Mg_i, the less responsive was the cell to insulin; 3) that IGF-I also stimulates Mg_i levels in erythrocytes; and 4) that this effect differs somewhat from that of insulin itself, because IGF-I was equally effective in stimulating Mg_i levels in cells from NT and HTN subjects in a manner not dependent on basal Mg_i levels, and preincubation of HTN cells with IGF-I partially reversed the blunted Mg_i responses to insulin. Together, these results suggest that IGF-I may contribute to regulate Mg_i levels, and that IGF-I induced increases in Mg_i levels may at least in part explain the previously described ability of IGF-I to enhance tissue insulin sensitivity.

Magnesium, the second most abundant intracellular cat-
ion, is involved in a number of important biochemical reactions, including all ATP transfer reactions. Possibly because of its relevance to all protein kinases, magnesium appears to mediate hormonal as well as other biochemical aspects of cellular glucose utilization (25). The intracellular magnesium deficiency demonstrated in insulin-resistant states such as hypertension and type II diabetes may thus contribute to suppressed glucose metabolism and insulin action (5–8). Conversely, insulin itself directly stimulates Mg<sub>i</sub> levels and may contribute to the regulation of Mg<sub>i</sub> levels (10, 11, 26). Thus, the shift from extra- to intracellular Mg observed in normal individuals after the ingestion of a glucose load was found to be smaller in type II diabetic patients, attributed to insulin resistance associated with NIDDM (27). Furthermore, insulin’s ability to increase levels of Mg was correlated with several parameters of insulin sensitivity (glucose uptake and disposal) and was negatively correlated with basal plasma insulin levels and excess body weight (28). Therefore, it is not clear to what extent the lower free magnesium levels in hypertension or diabetic syndromes (29, 30) directly contribute to insulin resistance, result from it, or both.

The ability of insulin to elevate cellular magnesium levels first reported by Lostroh (31, 32) has been also observed in erythrocytes and platelets (10, 11). Decreased magnesium responsiveness to insulin in cells from subjects with hypertension was demonstrated by measuring total magnesium content with atomic absorption spectroscopy (26, 27) and later by our group by measuring Mgi concentrations by NMR spectroscopy (33). Altered ionic actions of insulin in hypertension were linked with parallel alterations of insulin-mediated glucose uptake (27). These findings have been supported by similar recent studies of magnesium responses to insulin in NIDDM (34). Together, these results extend the concept of insulin resistance to include other, glucose-independent, ionic aspects of insulin action. Furthermore, altered magnesium responses to insulin may not necessarily be related to the hypertension per se, but may more generally reflect altered basal cellular magnesium levels. Indeed, as observed here, not only were insulin-induced changes in magnesium directly proportional to the initial Mgi level, but we have also reported previously that glucose disappearance after oral glucose loading is similarly directly related to basal in situ skeletal muscle Mg<sub>i</sub>; the lower the Mg<sub>i</sub>, the slower the fall in extracellular glucose (35). Additionally, depleting normal cells of magnesium also renders them insulin resistant (33). Hence, regardless of whether other primary abnormalities of insulin action exist in syndromes such as hypertension, these observations emphasize the potential contribution of altered cellular Mg<sub>i</sub> as an independent determinant of insulin action.

Although the effects of IGF-I on Ca<sub>i</sub> in different cellular systems have been investigated, i.e. cardiomyocytes (36), vascular smooth muscle cells (37), and osteoblasts (38), among others, the direct effects of IGF-I on cellular Mg metabolism observed here have not been previously reported. That these effects are independent of insulin is suggested by 1) the dissociation of Mg responses to insulin vis-à-vis IGF-I stimulation (in fact, for insulin, but not for IGF-I, cellular responsiveness depended on basal Mg levels); and 2) the ability of IGF-I to improve insulin-induced stimulation of Mg<sub>i</sub>. These results are consistent with the presence of independent receptors for insulin and IGF-I in erythrocytes (39, 40). The effects on Mg<sub>i</sub> of IGF-I vis-à-vis insulin also parallel the effects of these two peptides on glucose metabolism. Indeed, similar to our results in cells from HTN subjects, metabolic responses to IGF-I in insu-
lin-resistant diabetic rats were intact compared with impaired insulin-mediated effects on glucose uptake and intracellular glucose metabolism (41).

An interesting question is whether modifications in magnesium or other cation intake may alter basal Mg levels and therefore change the cellular Mg responsiveness to insulin. Two studies have demonstrated the ability of Mg supplementation to alter intracellular free Mg, values in NIDDM (42) and essential hypertension (43). Interestingly, in these reports the elevation of intracellular magnesium levels was paralleled with reduced platelet reactivity in response to a thromboxane A2 analog (42) and with decreased blood pressure and intracellular sodium (43). Future studies are needed to confirm the possible beneficial effects of magnesium supplementation on the ionic cellular environment as well as on the clinical manifestations of the associated conditions.

The ability of IGF-I to itself elevate Mg, in NT and HTN subjects may be clinically significant, as it would ameliorate the intracellular Mg deficiency that accompanies insulin-resistant states (6). Recently, IGF-I has been proposed as a therapeutic option in NIDDM and other insulin-resistant states, where it improves hyperglycemia despite an actual therapeutic option in NIDDM and other insulin-resistant states may be clinically significant, as it would ameliorate Mg supplementation to alter intracellular free Mg, values in NIDDM (42) and essential hypertension (43). In-terestingly, in these reports the elevation of intracellular magnesium levels was paralleled with reduced platelet reactivity in response to the thromboxane A2 analog (42) and with decreased blood pressure and intracellular sodium (43). Future studies are needed to confirm the possible beneficial effects of magnesium supplementation on the ionic cellular environment as well as on the clinical manifestations of the associated conditions.

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References


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