Insulin-Mimetic Action of Vanadate
Role of Intracellular Magnesium

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Abstract—The insulin-mimetic effect of vanadate is well established, and vanadate has been shown to improve insulin sensitivity in diabetic rats and humans. Although the exact mechanism(s) remain undefined, we have previously demonstrated a direct relation of intracellular free magnesium (Mg_i) levels to glucose disposal, to insulinemic responses following glucose loading, and to insulin-induced ionic effects. To investigate whether the insulin-mimetic effects of vanadate could similarly be mediated by Mg_i, we utilized 31P-nuclear magnetic resonance spectroscopy to measure Mg_i in erythrocytes from normal (NL, n=10) and hypertensive (HTN, n=12) subjects, before and after incubation with insulin and with different doses of sodium vanadate. In NL, vanadate elevated Mg_i levels, with maximum efficacy at 50 \( \mu \text{mol/L} \) (185±6 to 222±6 \( \mu \text{mol/L} \), \( P < 0.01 \)), as did physiologically maximal doses of insulin, 200 \( \mu \text{U/mL} \) (185±6 to 222±8 \( \mu \text{mol/L} \), \( P < 0.01 \)). In HTN, only vanadate, but not insulin, increased Mg_i (insulin: 173±7 to 180±9 \( \mu \text{mol/L} \), \( P = \text{NS} \); vanadate: 170±7 to 208±10 \( \mu \text{mol/L} \), \( P < 0.01 \)). Mg_i responses to insulin (50 \( \mu \text{mol/L} \), \( r = 0.637 \), \( P < 0.001 \)), but not to vanadate (50 \( \mu \text{mol/L} \), \( r = 0.743 \), \( P = \text{NS} \)), were closely and directly related to basal Mg_i levels. We conclude that (1) both vanadate and insulin stimulate erythrocyte Mg_i levels; (2) cellular Mg_i responses to insulin, but not to vanadate, depend on basal Mg_i content—the lower the basal Mg_i, the less the Mg_i response to insulin. As such, (3) Mg_i responses to vanadate were equivalent among HTN and NL, whereas HTN cells exhibited blunted Mg_i responses to insulin, and (4) the ability of vanadate to improve insulin sensitivity clinically may be mediated, at least in part, by its ability to increase Mg_i levels, which in turn, helps to determine cellular insulin action. (Hypertension. 2001;38[part 2]:701-704.)

Key Words: magnesium | vanadate | insulin | nuclear magnetic resonance spectroscopy

The phosphatase inhibitor vanadate is a compound that has insulin-mimetic properties both in vitro and in vivo, and it has been proposed in the treatment of diabetes mellitus.1 As such, vanadate lowers plasma glucose levels, increases peripheral glucose uptake, and improves insulin sensitivity.1,2

Depletion of another mineral ion, intracellular free magnesium (Mg_i), is a characteristic feature of insulin resistance in essential hypertension and type 2 diabetes mellitus, but it is not clear to what extent lower Mg_i levels contribute to insulin resistance, result from it, or both. Abnormalities of Mg_i and cytosolic free calcium (Ca_i) have been closely related to the level of blood pressure, the extent of cardiac hypertrophy, and the degree of insulin resistance present in these clinical states.3-6 Our group has proposed that the insulin resistance of hypertension and type 2 diabetes mellitus results, at least in part, from anionic defect characterized by cellular Mg_i deficiency and cellular Ca_i accumulation.5-7 We have previously demonstrated a direct relation of Mg_i levels to glucose disposal, to insulinemic responses following glucose loading, and to insulin-induced stimulation of Mg_i.5-8 Conversely, because the ability of insulin-like growth factor I (IGF-I) to improve insulin sensitivity may depend on its stimulation of Mg_i,9 we wondered whether the ability of vanadate to improve insulin action was also linked to cellular magnesium metabolism.

We therefore utilized 31P-nuclear magnetic resonance (NMR) spectroscopic techniques to assess Mg_i responses to vanadate compared with insulin in erythrocytes from normotensive and essential hypertensive individuals.

Subjects and Methods

Twenty milliliters of venous blood were drawn from unmedicated normotensive (n=10, male/female=5/5) and hypertensive (n=12, male/female=7/5) subjects in the morning (9:00 AM to noon) after an overnight fast. Previous medications were withdrawn for at least 3 weeks; diuretics, for at least 3 months before study. Essential hypertension was diagnosed on the basis of at least 3 blood pressure readings >150/90 mm Hg in the absence of signs or symptoms of secondary forms of hypertension. A history of myocardial infarction, angina pectoris, or stroke in the last 6 months before the study, as well as renal or hepatic failure, excluded the subject from consideration.

Cells from both normotensive and hypertensive subjects were incubated in the presence of varying doses of vanadate (1 to 1000 \( \mu \text{mol/L} \)) in a water bath maintained at 37°C, and Mg_i concentrations were measured serially over a period of 180 minutes. The vanadate dose inducing a maximal Mg_i response (50 \( \mu \text{mol/L} \)) was then
compared with a physiologically maximal dose of insulin (200 mU/mL) in separate experiments. Vanadate or insulin was directly added to the packed cells after centrifugation, immediately before the NMR measurement (see below). In another series of experiments \((n=8)\), vanadate (50 \(\mu\)mol/L) was added to the hypertensive cells after 60 minutes incubation with insulin (200 mU/mL). Insulin and vanadate actions on \(Mg_i\) were also tested, in cells from normotensive individuals, in the presence of a tyrosine kinase inhibitor (genistein, 100 \(\mu\)mol/L), and of a protein kinase inhibitor (staurosporin, 1 \(\mu\)mol/L).

### \(^{31}\)P-NMR Analysis of \(Mg_i\)

Erythrocyte \(Mg_i\) was measured according to a previously described method. Briefly, heparinized blood was centrifuged at 2000 rpm for 10 minutes, and the plasma was discarded. The remaining packed erythrocyte fraction was decanted into a 12-mm NMR tube, and after addition of vanadate or insulin, \(^{31}\)P-NMR spectra were recorded at 81 MHz at 37°C with a GE 300-MHz NMR spectrometer in the Fourier transform mode with wide-band proton noise decoupling. \(Mg_i\) levels were determined according to the formula \(Mg_i = K_d(MgATP) \times \Phi^{-1} - 1\), where \(\Phi\), the free, unbound fraction of ATP, is calculated from the chemical shift differences of the \(\alpha\)- and \(\beta\)-phosphoryl resonances of ATP in the \(^{31}\)P-NMR spectrum, and \(K_d(MgATP) = 38 \mu\)mol/L at 37°C.

### Statistical Analyses

The statistical significance of differences in \(Mg_i\) responses to each hormone treatment versus basal (no treatment) was estimated by ANOVA, using 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 with the CRUNCH software package on an IBM-compatible PC. All data are presented as mean±SEM.

### Results

Basal \(Mg_i\) levels were lower in erythrocytes from essential hypertensives compared with normotensive subjects (186±6 versus 171±7 \(\mu\)mol/L, \(P<0.05\)). In dose-response experiments, vanadate elevated \(Mg_i\) in erythrocytes from normotensive and hypertensive subjects at a threshold dose of 15 \(\mu\)mol/L, and a maximal effect was observed at 50 \(\mu\)mol/L (Figure 1). In the time course experiments, the effect of vanadate was observed at 30 minutes and peaked at 60 minutes of incubation both in normotensive subjects and in hypertensive subjects (\(P<0.05\) at each time versus basal) (Figure 2).

In contrast, insulin’s effect to stimulate \(Mg_i\) levels was present in cells from normotensive subjects but not in cells from hypertensive subjects (Figure 3). Vanadate (50 \(\mu\)mol/L) also significantly increased \(Mg_i\) in insulin-treated cells from hypertensive subjects, in whom insulin alone (200 mU/mL) did not alter \(Mg_i\) levels (basal \(Mg_i\): 176±8 \(\mu\)mol/L; \(Mg_i\) after addition of insulin: 182±9 \(\mu\)mol/L, \(P=NS\); \(Mg_i\) after sequential addition of vanadate: 203±8 \(\mu\)mol/L, \(P<0.05\)). In cells from normal subjects, staurosporin or genistein inhibited the insulin-induced \(Mg_i\) increase (from 179.8±1.3 to 180.9±1.6 \(\mu\)mol/L, \(P=NS, n=6\)) but did not alter the ability of vanadate to stimulate \(Mg_i\) (from 177.0±1.5 to 210.0±3.9 \(\mu\)mol/L, \(P<0.001, n=6\)).

The different \(Mg_i\) responses to vanadate versus insulin in hypertensive versus normotensive cells were true both for maximal absolute \(Mg_i\) levels attained and for the change in hypertensive subjects, in whom insulin alone (200 mU/mL) did not alter \(Mg_i\) levels (basal \(Mg_i\): 176±8 \(\mu\)mol/L; \(Mg_i\) after addition of insulin: 182±9 \(\mu\)mol/L, \(P=NS\); \(Mg_i\) after sequential addition of vanadate: 203±8 \(\mu\)mol/L, \(P<0.05\)). In cells from normal subjects, staurosporin or genistein inhibited the insulin-induced \(Mg_i\) increase (from 179.8±1.3 to 180.9±1.6 \(\mu\)mol/L, \(P=NS, n=6\)) but did not alter the ability of vanadate to stimulate \(Mg_i\) (from 177.0±1.5 to 210.0±3.9 \(\mu\)mol/L, \(P<0.001, n=6\)).

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Mg, from baseline values. For all subjects, regardless of diagnostic clinical blood pressure category, the cellular Mg responsiveness to insulin was closely linked to basal Mg, \( r = 0.637, P < 0.01 \): the lower the basal Mg level, the more blunted the cell Mg response (Figure 4A). This was not true for vanadate, for which stimulation of Mg occurred independently of basal Mg levels in normotensive and hypertensive subjects \( r = 0.15, P = \text{NS} \) (Figure 4B).

**Discussion**

Magnesium, the second most abundant intracellular cation, 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. The Mg deficiency that has been demonstrated in insulin-resistant states such as hypertension and type 2 diabetes may thus contribute to suppressed glucose metabolism and insulin action.

The insulin-mimetic action of vanadate on glucose homeostasis and peripheral tissues has been well established both in vitro and in vivo. In animal models of diabetes, vanadate treatment decreases hyperglycemia and insulin levels and improves tolerance to oral glucose. In humans, vanadate increases nonoxidative glucose disposal, as measured by indirect calorimetry, and decreases insulin requirements in diabetic patients, suggesting that it may have a potential role as adjunctive therapy in patients with diabetes mellitus.

Our approach here was to compare the ionic effects of insulin with those of vanadate, the effect of which on Mg levels has not previously been studied. We observed that (1) insulin at physiologically maximal concentrations significantly elevates Mg levels in cells from normotensive subjects, but not in cells from hypertensive subjects; (2) for all subjects, independently of their designation as normotensive or hypertensive, Mg responsiveness to insulin was closely and directly related to basal Mg levels—the lower the basal Mg, the less responsive was the cell to insulin; (3) vanadate also stimulates Mg levels in erythrocytes; and (4) this vanadate effect differs somewhat from that of insulin itself, because (a) vanadate was equally effective in stimulating Mg levels in cells from normotensive and hypertensive subjects, in a manner not dependent on basal Mg levels, and (b) incubation of insulin-treated hypertensive cells with vanadate significantly stimulated Mg.

The NMR techniques we used here possess certain advantages, including greater precision and reproducibility, and preserving conditions that closely represent the in situ extracellular environment. However, our data obtained in red blood cells need to be confirmed in other tissues, such as vascular smooth muscle.

Although other ionic actions of vanadate in erythrocytes have previously been demonstrated, vanadate increasing Ca, the direct effect of vanadate on cellular magnesium metabolism observed here has not previously been reported. That these effects are independent of insulin is suggested by (1) the dissociation of Mg responses to insulin vis-à-vis vanadate stimulation. In fact, for insulin, but not for vanadate, cellular responsiveness depended on basal Mg levels and (2) the ability of vanadate to improve insulin-induced stimulation of Mg.

Although our present experiments cannot fully elucidate the causal mechanism(s) involved, the Mg effects of vanadate vis-à-vis insulin suggest different mechanisms (and/or sites of action) of Mg stimulation by vanadate and insulin. Our present results are in agreement with data in the literature that show vanadate does not alter basal and insulin-stimulated receptor kinase activities, suggesting that the corrective effect of vanadate on magnesium and glucose metabolism may be distal to insulin receptor kinase activity, which is a magnesium-dependent enzyme. Interestingly, the phosphatases that are inhibited by vanadate are all magnesium independent. We suggest, therefore, that the magnesium dependency of insulin-dependent kinases may account for the blunting of insulin action in magnesium-depleted cells (Figures 3 and Figure 4A) and may also explain the dissociation of vanadate action from cellular magnesium content (Figure 4B). This is also supported by the experiments with the kinase inhibitors genistein and staurosporin, which inhibited the magnesium-stimulating action of insulin but not of vanadate. Thus, the ability of vanadate to itself elevate Mg in normotensive and hypertensive subjects may be clinically significant and may account at least in part for its ability to improve insulin sensitivity. By increasing Mg, vanadate would offset the decreased Mg-dependent, insulin-stimulated phosphorylation of signaling peptides present in insulin resistance and thereby improve glucose transport.

Other reported effects of vanadate also need to be considered. In vitro incubation with vanadate at concentrations ranging from 20 to 80 \( \mu \text{mol/L} \) may increase smooth muscle contractility and acute administration of vanadium compounds is known to elevate blood pressure. In support of these findings, in vitro incubation with vanadate was shown to increase CA in rat aortic smooth muscle cells with an \( EC_{50} \) value of \( \approx 42 \mu \text{mol/L} \), and vanadate may increase tension in...
rat aortic strips. However, vanadate (in 40 to 80 μmol/L range) may also promote endothelial-dependent vasodilation, which may reduce systolic blood pressure in spontaneously hypertensive rats and may also ameliorate the exaggerated vasoconstriction in aortic tissue from the hyperinsulinemic/insulin-resistant obese Zucker rat. McNeill and coworkers have shown that long-term vanadium treatment of insulin-resistant/diabetic rats led to reduction in BP, perhaps related to normalization of metabolic alterations in the insulin-resistant and diabetic state by vanadium compounds. The changes in erythrocyte Mg levels may be intimately linked to changes in erythrocyte Ca levels. In addition to alterations in Ca, levels via activation of PLC-γ and as a result of protein tyrosine phosphatase inhibition, via increased tyrosine kinase activity, vanadate at concentration ranges used in this study (50 μmol/L) could also affect sodium-pump activity. Finally, vanadate may promote hemolysis at high concentrations, and this may account for the low Mg values seen at high vanadate concentrations (Figure 1). Altogether, the present study supports the overall hypothesis that the intracellular ionic milieu is at least 1 determinant of cellular responsiveness to insulin, and that vanadate alters Mg homeostasis. Future studies are needed to elucidate in greater mechanistic detail how vanadate action on cellular magnesium metabolism contributes to its insulin-mimetic and/or vascular actions.

References

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