L-Glutamic Acid γ-Monohydroxamate

A POTENTIATOR OF VANADIUM-EVOKED GLUCOSE METABOLISM IN VITRO AND IN VIVO*

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We report that the vanadium ligand L-Glu(γ)HXM potentiates the capacity of free vanadium ions to activate glucose uptake and glucose metabolism in rat adipocytes in vitro (by 4–5-fold) and to lower blood glucose levels in hyperglycemic rats in vivo (by 5–7-fold). A molar ratio of two L-Glu(γ)HXM molecules to one vanadium ion was most effective. Unlike other vanadium ligands that potentiate the insulinomimetic actions of vanadium, L-Glu(γ)HXM partially activated lipogenesis in rat adipocytes in the absence of exogenous vanadium. This effect was not manifested by D-Glu(γ)HXM. At 10–20 μM L-Glu(γ)HXM, lipogenesis was activated 9–21%. This effect was approximately 9-fold higher (140 ± 15% of maximal insulin response) in adipocytes derived from rats that had been treated with vanadium for several days. Titration of vanadium(IV) with L-Glu(γ)HXM led to a rapid decrease in the absorbance of vanadium(IV) at 765 nm. and 51V NMR spectroscopy revealed that the chemical shift of vanadium(IV) at ~490 ppm disappeared with the appearance of a signal characteristic to vanadium(V) (~530 ppm) upon adding one equivalent of L-Glu(γ)HXM. In summary, L-Glu(γ)HXM is highly active in potentiating vanadium-activated glucose metabolism in vitro and in vivo and facilitating glucose metabolism in rat adipocytes in the absence of exogenous vanadium probably through conversion of trace intracellular vanadium into an active insulinomimetic compound. We propose that the active species is either a 1:1 or 2:1 L-Glu(γ)HXM vanadium complex in which the endogenous vanadium(IV) has been altered to vanadium(V). Finally, we demonstrate that L-Glu(γ)HXM- and L-Glu(γ)HXM-vanadium-evoked lipogenesis is arrested by wortmannin and that activation of glucose uptake in rat adipocytes is because of enhanced translocation of GLUT4 from low density microsomes to the plasma membrane.

Intensive studies have been carried out during the last two decades on the insulinomimetic effects of vanadium (1–4). Vanadium salts mimic most of the effects of insulin on the main target tissues of the hormone in vitro and also induce normoglycemia and improve glucose homeostasis in insulin-deficient (5–7) and insulin-resistant diabetic rodents (in vivo) (5–8). On the basic research frontier, data continue to accumulate showing that vanadium salts manifest their insulin-like metabolic effects through alternative pathways not involving insulin receptor tyrosine kinase activation or phosphorylation of insulin receptor substrate 1 (9–19). The key events of this backup system appear to involve inhibition of protein-phosphotyrosine phosphatases and activation of nonreceptor protein-tyrosine kinases (20–23).

Vanadium salts are seriously considered as a possible treatment for diabetes, and several clinical studies have already been performed. In those studies, because of its toxicity, only low doses of vanadium (2 mg/kg/day) were used. Although ~20-fold lower than doses used in most animal studies, several beneficial effects were observed and documented (24–26). Any manipulation to elevate the insulinomimetic efficacy of vanadium without increasing its toxicity is of major clinical interest for the future care of diabetes (reviewed in Ref. 27).

Organically chelated vanadium compounds, such as vanadium-acetylacetonate and vanadium-RL-252,1 are more potent than free vanadium in facilitating insulin-like effects in rat adipocytes (28, 29). Similarly, chelated vanadium compounds such as bis(maltolato)oxovanadium and bis(picolinato)oxovanadium are more effective than free vanadium in reducing circulating glucose levels in hyperglycemic streptozocin-treated rats (30–33).

In the wake of these findings, we have continued our search for more effective vanadium binding agents. Of special interest to us were vanadium chelators that synergize with vanadium both in vitro (i.e. in streptozocin rats) and in vivo (i.e. in isolated rat adipocytes) and therefore enable us to gain insight into the basic mechanism(s) by which such compounds potentiate the insulinomimetic activity of vanadium. Specifically, we have studied hydroxamic acid derivatives. These compounds are involved in the microbial transport of iron and are therefore applied therapeutically in conditions of iron deficiency (34). They are also inhibitors of urease activity and have been used in the treatment of hepatic coma. Monoamino acid hydroxamates are simple, nontoxic derivatives of amino acids. D-Aspartic acid β-hydroxamate was shown to have antitumoral activity on murine leukemia L5178Y, both in vitro and in vivo, and is

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1 The abbreviations used are: RL-252, [(CH2)n-C-(CH2)n-CO-NH]H{BuCONOHCH(C)H}l; L-Glu(γ)HXM, L-glutamic acid γ-monohydroxamate; GLUT4, glucose transporter 4; PM, plasma membranes; LDM, low density microsomes; BSA, bovine serum albumin; VOCl2, vanadyl dichloride; NaVO2, sodium metavanadate.
active against Friend leukemia cells in vitro as well (35). L-Glu(γ)HXM is cytotoxic against L1210 cells in culture and remarkably antitumoral against L1210 leukemia and B16 melanoma in vivo (35, 36).

EXPERIMENTAL PROCEDURES

Materials—D-[U-14C]glucose and 2-deoxy-D-[G-3H]glucose were purchased from NEN Life Science Products. Collagenase type I (134 units/mg) was obtained from Worthington. Porcine insulin was purchased from Eli Lilly Co. (Indianapolis, IN). Phloretin, 2-deoxyglucose, L-glutamic acid(γ)-monohydroxamate, L-aspartic acid(β)-monohydroxamate, glycine hydroxamate, L-isoleucine(α)-hydroxamate, and L-tyrosine(α)-hydroxamate were purchased from Sigma. RL-252 was prepared and characterized as described earlier (28).

Krebs-Ringer bicarbonate (KRB) buffer (pH 7.4) contained 110 mM NaCl, 25 mM NaHCO3, 5 mM KCl, 1.2 mM KH2PO4, 1.3 mM CaCl2, 1.3 mM MgSO4, 4 mM KH2PO4, 30 mM HEPES. All other chemicals and reagents used in this study were of analytical grade.

Streptozocin-treated Rats—Diabetes was induced by a single intravenous injection of a freshly prepared solution of streptozocin (55 mg/kg body weight) in 0.1 M citrate buffer, pH 4.5 (9). The effect of the L-Glu(γ)HXM-vanadium complex on blood glucose level was determined 8 days after induction of diabetes by streptozocin.

Cell Preparation and Bioassays—Rat adipocytes were prepared from the fat pads of male Wistar rats (130–150 g) by collagenase digestion according to the method of Rodbell (37). Cell preparations showed more than 95% viability by Trypan blue exclusion at least 3 h after digestion. All bioassays were performed as described in figure legends. Glucose transport was carried out using 2-deoxy-D-[G-3H]glucose uptake (38), and lipogenesis (the incorporation of U-14C-labeled glucose into lipids) was performed according to Moody et al. (39). Briefly, freshly prepared rat adipocytes were suspended in KRBH buffer and divided into about 50 plastic vials. Each vial contained 0.5 ml of adipocyte suspension (about 1.5 × 10^5 cells). These were incubated for 2 h at 37 °C under an atmosphere of 95% O2, 5% CO2 with 0.16 mM [U-14C]glucose. Each assay contained vials with and without 0.3 nM insulin and the various test compounds. Lipogenesis was terminated by adding toluene-based scintillation fluid, and the extracted lipids were counted (39). Results are expressed as a percent of maximal insulin response. Only assays in which insulin activated lipogenesis 5–6-fold above basal (basal; 4000 cpm/1.5 × 10^5 cells/2 h, Vmax = 20,000–24,000 cpm/1.5 × 10^5 cells/2 h) were taken into consideration. Insulin activated lipogenesis in this assay at an ED50 value of 33 ± 6 pM. A concentration of 0.3 nM insulin and above already facilitated maximal (100%) response (i.e., Ref. 16). All assays were performed in duplicate or triplicate.

Western Immunoblot Analysis of GLUT4 in Subcellular Membranes Following Stimulation of Rat Adipocytes—Adipocytes prepared from 6-week-old rats were incubated with and without insulin and with L-Glu(γ)HXM alone and complexed with vanadate as specified in the figure. Cells were then homogenized and fractionated to low density microsomal membrane (LDM) and plasma membrane (PM) fractions by differential ultracentrifugation according to Ref. 40. Membrane proteins were then solubilized in sample buffer for 30 min at 25 °C, resolved on 10% SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose paper, and immunoblotted with anti-GLUT4 antisera (41). Visualization was performed by phosphoimaging. The relative intensity of bands corresponding to GLUT4 was quantitated using MacBas 1000.

51V NMR Spectroscopy—The 51V NMR spectra were recorded on a 200-MHz Bruker WPS4 (4.7T) spectrometer. Spectrum width of 16,000
H₃O₂ a 90° pulse angle, and an accumulation time of 0.28 were used. The chemical shifts are reported relative to the external reference standard VOCl₂ (490 ppm).

RESULTS

L-Glutamic Acid(γ)Monohydroxamate Potentiates Vanadium-evoked Lipogenesis in Rat Adipocytes—In this set of experiments, rat adipocytes were incubated for 10–20 min with sub maximal concentrations of vanadate (10–30 μM), L-Glu(γ)HXM (10–30 μM), or an equimolar combination of them. The capacity to activate lipogenesis relative to insulin was then determined. As shown in Fig. 1, the combination was highly synergistic. For example, at 10 μM vanadate or L-Glu(γ)HXM, lipogenesis was 17 ± 3 and 9 ± 2%, respectively, whereas the combination produced a marked incredible 93 ± 4% activation of maximal insulin response. At 25 μM, the extent of lipogenesis was 37 ± 3, 20 ± 3, and 121 ± 7%, and at 30 μM, it was 42 ± 4, 23 ± 4, and 143 ± 7% of maximal. Wortmannin (100 nM), an inhibitor of phosphatidylinositol 3-kinase, fully blocked the activating effects of vanadate, L-Glu(γ)HXM, and its combination with vanadate Fig. 1, right columns). Thus L-Glu(γ)HXM potentiates vanadate-evoked- lipogenesis about 3.5–5-fold; the higher concentrations reached a level that is about 140% of that achieved by saturating concentrations of insulin or vanadate. A finding of significant interest to us was the ability of L-Glu(γ)HXM to partially activate lipogenesis even in the absence of exogenous vanadium (Fig. 1). This finding is examined in great detail in connection with Fig. 6.

In Fig. 2, lipogenesis in rat adipocytes was evaluated at a fixed, low concentration of vanadate (5 μM) with increasing concentrations of L-Glu(γ)HXM. Lipogenesis was negligible at 5 μM vanadate or L-Glu(γ)HXM alone (4–6% of maximal insulin effect) but is augmented to 27.0 ± 3% when they were given in combination (at a molar stoichiometry of 1:1). At 2:1 and 3:1 Glu/γHXM-vanadium molar stoichiometry, lipogenesis expanded to 43 and 57%, respectively, of maximal response. Thus a substantial synergistic effect is obtained at a 1:1 molar ratio and is increased further at a 2:1 molar stoichiometry and even higher, though much less pronounced, at a 3:1 molar ratio (Fig. 2).

L-Glu(γ)HXM Potentiates Vanadate-evoked Glucose Uptake—Fig. 3 shows activation of 2-deoxyglucose uptake by low concentrations of vanadate (20 μM), L-Glu(γ)HXM (40 μM), and by the 2:1 molar combination of them. 2-Deoxyglucose undergoes insulin- or vanadate-evoked influx into the cell via the same transporters as glucose and is phosphorylated in situ to 2-deoxyglucose-6-phosphate with no further metabolism (42, 43). Therefore, this measurement reflects an effect on glucose entry into the cell in a manner largely independent of the metabolism of the endogenous saccharide. Vanadate (20 μM) and L-Glu(γ)HXM (40 μM) affected 2-deoxyglucose uptake of 7 ± 0.7 and 31 ± 4% of maximal insulin effect, respectively. Together they caused 2-deoxyglucose uptake 117 ± 9% of maximal insulin response (Fig. 3).

L-Glu(γ)HXM Alone and L-Glu(γ)HXM-Vanadate Lead to Translocation of GLUT4 from LDM to PM Fractions in Rat Adipocytes—Incubation of rat adipocytes with L-Glu(γ)HXM and L-Glu(γ)HXM-vanadate led to a decrease in the content of GLUT4 in the LDM fraction and an increase in the PM fraction (Fig. 4). The decrease in GLUT4 content in the low density lipoprotein fraction amounted to 32 ± 3 ± 1, and 68 ± 5% of maximal insulin response upon incubating the cells with L-Glu(γ)HXM (40 μM), vanadate (20 μM, not shown), and the combination, respectively (calculated from Fig. 4). Under similar experimental conditions, L-Glu(γ)HXM, vanadate, and the combination activated 2-deoxyglucose uptake to an extent of 31 ± 4, 7 ± 0.7, and 117 ± 9% of maximal insulin response (Fig. 3), suggesting a contributing effect of the complex to glucose influx in addition to its effect in recruiting GLUT4 transporters...
from the low density lipoprotein to the PM fraction.

Streptozocin-treated Diabetic Rats—In the experiments summarized in Fig. 5, streptozocin-treated rats received intraperitoneally sodium metavanadate (0.05 mmol/kg body weight), L-Glu(γ)HXM (0.1 mmol/kg body weight), or a combination of the two compounds 8 days after the induction of diabetes. As shown in the figure, vanadate and L-Glu(γ)HXM, at these concentrations, had a rather minor effect in reducing the high circulating glucose levels characterizing these hyperglycemic rats. The combination, however, was highly efficient at normalizing blood glucose levels. Normoglycemia was evident 1 day after the first administration and remained so following two more administrations. The glucose levels then remained close to normal for the next 3 days (Fig. 5).

Activation of Lipogenesis in Rat Adipocytes by L-Glu(γ)HXM in the Absence of Exogenous Vanadium—L-glutamic acid(γ)HXM also activated lipogenesis in the absence of added vanadium, and this effect was studied in detail (Fig. 6). The dose-response curve (Fig. 6A) indicates that activation is already evident at 5 μM L-Glu(γ)HXM and that higher concentrations reach a level of 40–67% of maximal insulin response (median effective dose 5.35 ± 35 μM). Other amino acid hydroxamates such as L-Tyr(α)HXM, Gly(α)HXM, and L-Ile(α)HXM also activated lipogenesis, but they were considerably less potent (ED₅₀ = 250 ± 30 μM, 40 ± 5% of maximal insulin effect). L-Aspartic acid β-monohydroxamate showed higher lipogenic activity compared with the α-amino acid hydroxamates and was slightly less potent than L-Glu(γ)HXM (ED₅₀ = 45 ± 7 μM, Fig. 6B). N-acetyl-L-Glu(γ)HXM and L-Glu(γ)HXM-α-methyl ester were virtually ineffective, indicating the need for a free α-amino and, to a somewhat lesser extent, a free

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α-carboxyl moiety for the activation of lipogenesis by L-Glu(γ)HXM in the rat adipose cell (Fig. 6C). Stereospecificity appears crucial as well, because the D-isomer of Glu(γ)HXM was ineffective. All these findings indicate that activation of lipogenesis by L-Glu(γ)HXM depends on a specific entry of this L-amino acid analog into the adipose cell. Further investigation has led us to suggest that L-Glu(γ)HXM enters the adipose cell primarily through the non-Na⁺-dependent glutamine transport system.²

Several organic chelators, which potentiate the insulinomimetic activity of vanadium either in vitro or in vivo, have been documented. These include acetylacetone (29), maltol (30, 31), picolinate (32, 33), and RL-252 (28). In Fig. 6D, we have examined whether they are capable of activating lipogenesis in the absence of exogenous vanadium. Unlike L-Glu(γ)HXM, none of these agents were able to activate lipogenesis in the rat adipose cell at concentrations of 100 μM (Fig. 6D) or lower (not shown).

Extensive Potentiation of L-Glu(γ)HXM-evoked Lipogenesis in Rat Adipocytes in Vitro Following Enrichment with Vanadium in Vivo—The findings presented in Figs. 1–4 have taught us that L-Glu(γ)HXM potentiates the insulinomimetic potency of vanadium and that activation of lipogenesis by L-Glu(γ)HXM alone never exceeds 40 ± 7% of maximal insulin effect (Fig. 6).

To examine whether L-Glu(γ)HXM-evoked lipogenesis can be affected by the level of intracellular vanadium, a group of male Wistar rats received daily subcutaneous administrations of vanadate (0.1 mmol/kg/day) over a period of 5 days to raise the level of endogenous vanadium. Rats were then sacrificed 7 h after the last administration. Adipocytes were prepared, and the effect of L-Glu(γ)HXM on lipogenesis was compared with that in nontreated freshly prepared adipocytes. As shown in Fig. 7, vanadium-enriched adipocytes became dramatically sensitive to L-Glu(γ)HXM-evoked lipogenesis. This was valid both in terms of a leftward shift in the dose-response curve to L-Glu(γ)HXM (ED₅₀ = 6.4 ± 0.3 μM versus ED₅₀ = 35 ± 4 μM in control adipocytes) and in terms of the degree of lipogenesis (145 ± 15 versus 40 ± 7% of maximal insulin response, i.e. Fig. 6). At 10 μM, L-Glu(γ)HXM already stimulated lipogenesis and amounted to 120% of maximal insulin effect in the vanadium-enriched adipose cells (as opposed to only 8.0 ± 1.5% in control adipocytes) (Fig. 7).
Spectroscopic Studies—Previously we found in cell-free experiments that vanadium(IV), at neutral pH values, undergoes slow spontaneous oxidation to vanadium(V). This occurs similarly in the presence of 10 mM reduced glutathione, an ineffective reductant of vanadium(V), at neutral pH values with a $t_{1/2}$ value of 1.6 hours at 25°C (29). The results summarized in Fig. 8 show the $^{51}$V NMR spectra of vanadium(V), vanadium(IV), and a mixture of vanadium(IV) with L-Glu($\gamma$)HXM. Vanadium(IV) appeared as a single peak with a chemical shift of 2490 ppm in its $^{51}$V spectrum, indicating one main species present at >95% purity. Upon the addition of L-Glu($\gamma$)HXM (1 equivalent), the chemical shift of vanadium(IV) at ~490 ppm disappeared within minutes and the principal chemical shift characterizing vanadium(V) at ~530 ppm appeared (Fig. 8).

Vanadium(IV) (i.e. vanadyl sulphate or VOCl$_2$) has a characteristic "blue" absorbance with $\varepsilon_{765\,\text{nm}} = 14 \pm 0.3$, whereas vanadium(V) does not absorb at all at this wavelength (29). The addition of 2–3 equivalents of L-Glu($\gamma$)HXM to VOCl$_2$(IV) (50 mM at pH 7.5) led rapidly to a near total decrease in vanadium(IV) absorbance at 765 nm (Fig. 9). Fig. 8D depicts complex formation as a function of the pH in the range of pH 2–9. Decrease is minimal at pH 4.0, quite significant at pH 5.0,
half-maximal at pH 5.7, and reaches a stable plateau at pH range 7–9 (Fig. 9B).

DISCUSSION

It has been consistently observed that chelated vanadium compounds are more potent than the free metal oxide in facilitating the metabolic actions of insulin. This was demonstrated in vitro with systems like rat adipocytes, as well as in diabetic rodents such as streptozocin-treated hyperglycemic rats (28–33). Because of the variations in the experimental models used, the oxidation state of vanadium applied, and the different administration modes, the basis for the higher insulinomimetic potencies of complexed vanadium remained rather speculative. Because this topic has immediate therapeutic relevance, we looked for new vanadium chelators characterized by: (a) higher synergistic potencies than previously documented for vanadium chelators with respect to vanadium-evoked glucose uptake and glucose metabolism both in vitro and in diabetic rats in vivo, (b) low indices of toxicity, and (c) reasonable solubility in aqueous, neutral media after complexation with vanadium.

In this study, we have introduced the L-isomer of glutamic acid(γ)monohydroxamate as it satisfactorily fulfilled the above criteria. It potentiated vanadium-activated hexose uptake, glucose metabolism, and recruitment of GLUT4 transporters from LDM to PM fractions (Figs. 1–4). In vivo it potentiated the efficacy of vanadium to lower blood glucose levels in streptozocin rats (Fig. 5). This amino acid analog has negligible toxicity in mammals. Both L-Glu(γ)HXM alone and its complexes with vanadium are fairly soluble in aqueous media at neutral pH values. An important finding was that L-Glu(γ)HXM alone, in the absence of exogenous vanadium, showed a reasonable amount of insulinomimetic activity in that it activated glucose uptake and glucose metabolism in the rat adipose cell (Figs. 1–3). Further investigation revealed that this activating effect is unique to the L-isomer of Glu(γ)HXM but is not facilitated by the D-isomer. Nonmodified α-amino and α-carboxyl moieties appear essential. This intrinsic activity is exclusive to L-Glu(γ)HXM not being shared by any of the other vanadium chelators that potentiate the actions of vanadium in vivo or in vitro (Fig. 6, A–D, and Refs. 28–33). Our assumption that L-Glu(γ)HXM permeates into the cell interior and transforms the “dormant” intracellular vanadium pool into an insulinomimetic-activated species gains credence from the dramatic sensitization of vanadium-enriched adipocytes to L-Glu(γ)HXM-evoked lipogenesis (Fig. 7).

It should be mentioned at this point that because of the extreme complexity of aqueous vanadium chemistry (reviewed in Refs. 46–49), the intracellular milieu of the mammalian cell is still “a black box” with respect to the state and the form of entered vanadium. With the endogenously present vanadium pool, experiments have shown that it exists mostly as vanadium(IV), though some researchers may wonder even about this experimental finding because vanadium in its IV oxidation state is only stable at acidic pH values (pH < 3.0) and readily oxidizes to vanadium(V) at neutral pH even in the presence of high glutathione concentrations (28, 46). The intracellular vanadium pool, however, can be preserved in its IV oxidation form at neutral pH values if it is chelated by ascorbic acid (not shown) or to endogenous proteins (50, 51). At the low physiological level of intracellular vanadium, the cell should have the capacity to chelate all the endogenous vanadium.

Our experimental findings that L-Glu(γ)HXM alone enhances glucose uptake and glucose metabolism (Figs. 1 and 2) together with the apparent rapid conversion of vanadium(IV) to vanadium(V) upon complexion (Figs. 8 and 9) strongly support the contention that vanadium(V) rather than vanadium(IV), and in a chelated form, is the active insulinomimetic species that facilitates the activation of glucose uptake and its metabolism in rat adipocytes. Although most of our previous cell-free experiments support this conclusion, we were not fully convinced prior to the completion of this study. This is because protein phosphotyrosine phosphatases (with p-nitrophenylphosphate as a substrate) are inhibited by both vanadium(IV) and vanadium(V), free or chelated, at nearly the same concentrations (see Ref. 52). On the other hand, adipose nonreceptor protein-tyrosine kinases, whether cytosolic or membranal, are with one exception activated by vanadium(V) but not at all by vanadium(IV) (22, 23). We have only observed vanadium(IV)-evoked activation of nonreceptor protein-tyrosine kinases when membranal protein phosphotyrosine phosphatases were extracted with Triton X-100 and added to the cytosolic protein-tyrosine kinase fraction (29). These experimental conditions, however, are not likely to occur in the intact cell system. For example, broken plasma membrane fragments (or deoxycholate-treated membranal fragments) did not sup-

![Fig. 9. Decrease in absorbance of vanadium(IV) at 765 nm upon addition of L-Glu(γ)HXM. Effect of pH. A, left column, absorbance of VOCl₂ (50 mM) in H₂O, VOCl₂ (50 mM) and L-Glu(γ)HXM (150 mM) titrated with NaHCO₃ to pH 7.4. B, right column, absorbance of VOCl₂ (50 mM) and L-Glu(γ)HXM (100 mM) in H₂O were titrated either with HCl or with NaHCO₃ before absorbance at 765 nm and were monitored to obtain the pH values indicated in the figure. L-Glu(γ)HXM alone does not absorb at 765 nm. Vanadium dichloride alone, which tends to precipitate at neutral pH values, remains completely soluble at all pH values in the presence of two or more equivalents of L-Glu(γ)HXM.](image-url)
port activation of cytosolic protein-tyrosine kinases in the presence of vanadium(IV) (29).

In summary, L-Glu(γ)HXM appears superior to previously documented organic chelators of vanadium in potentiating its activation of glucose uptake and glucose metabolism in vitro and in vivo. Taken together with earlier studies, this may be attributed to one or more of the following: (a) increased efficiency of this specific combination to permeate into cells or tissues; (b) a favorable 5-coordinated, rather than octahedral topography of this complex in an aqueous, neutral environment (Ref. 50); and/or (c) higher intracellular stability of the L-Glu(γ)HXM-vanadium complex. Finally, we have recently observed that vanadate does not inhibit alkaline phosphatase in the presence of L-Glu(γ)HXM. This inhibitory effect of vanadate (53) is undesirable from our point of view as it may contribute to vanadium toxicity in mammals, but not to the efficacy of vanadium to manifest the metabolic actions of insulin (reviewed in Ref. 54). This and other basic and diabetological aspects raised here are being further investigated.

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