Vanadium Compounds as Insulin Mimics

Katherine H. Thompson, John H. McNeill, and Chris Orvig*

Medicinal Inorganic Chemistry Group, Chemistry Department, and Faculty of Pharmaceutical Sciences, The University of British Columbia, Vancouver, British Columbia, Canada V6T 1Z1

Received February 2, 1999 (Revised Manuscript Received May 31, 1999)

Contents

I. Introduction

Vanadium, element number 23, atomic weight 50.94, is normally present at very low concentrations ($<10^{-8}$ M) in virtually all cells in plants and animals. Vanadium in oxidation states III, IV, and V readily forms V−O bonds and comfortably binds N and S as well, forming chemically robust coordination complexes. Vanadium(V), in particular, is stereochemically flexible—coordination geometries ranging from tetrahedral and octahedral to trigonal pyramidal and pentagonal bipyramidal are thermodynamically plausible.[1] The potential for redox interplay, whether V(V)/V(IV) or V(IV)/V(III), increases the versatility of this element in the biological milieu.[2] Although not a common component of enzymes, vanadium as the vanadate ion is an essential prosthetic group of some haloperoxidases,[3] which are currently being elucidated in great detail.[4] Vanadium may or may not play an essential role in normal mammalian metabolism;[5] however, at pharmacological concentrations, as a potential therapeutic agent, it is attracting increasing attention (for recent reviews, see refs 6−8).

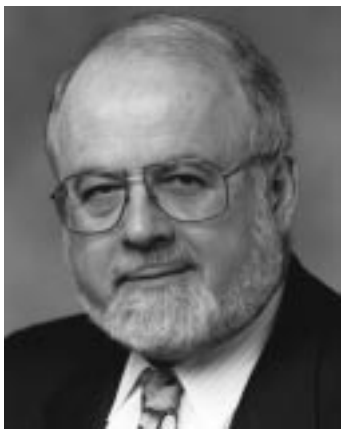
Three general classes of vanadium-containing compounds are of interest for their utility as insulin-mimetic agents: (1) inorganic vanadium salts, both anionic (vanadates $[VO_4]^{3-}$) and cationic (vanadyl VO^{2+}), (2) complexes resulting from combination of vanadium(V) and hydrogen peroxide (mono- and diperoxovanadates, $[VO(O_2)(H_2O)_2(L-L')]^{n-}$ ($n = 0,1$) and $[VO(O_2)_2(L-L')]^{n-}$ ($n = 1, 2, 3$), and (3) chelated vanadium(IV) complexes.[9] In this review, the principal focus will be on the chemistry of those vanadium and peroxovanadium complexes deemed to have potential as insulin-mimetic agents in the treatment of human diabetes mellitus.

II. Insulin in Glucose and Fat Metabolism

The fact that chemical entities are being developed as insulin mimics does not presuppose completely symmetrical functionality. Insulin is a signaling hormone which is essential both for fat and carbohydrate metabolism. It is secreted by the pancreas in response to elevated levels of glucose in the bloodstream, at levels which occur naturally following ingestion of a meal. The increased insulin then normally promotes glucose uptake by the liver and gut (splanchnic tissues), as well as by peripheral tissues (adipose and muscle), which results in energy production and storage, as needed, by the organism.[10] This promotion of glucose uptake (and associated inhibition of lipolysis) in a tissue-specific manner can be duplicated by vanadium compounds.[11] Insulin also serves to counteract catabolic hormones, such as glucagon, and to suppress production of glucose in the liver, effects which are not always replicated by vanadium.[12] Vanadium compounds can never completely substitute for insulin; however, many of the observed in vitro and in vivo effects are insulin-like.[6,7] We use the term insulin mimic in this more general sense throughout our review.

10.1021/cr980427c CCC: $35.00
Published on Web 08/07/1999

Katherine Thompson completed her undergraduate studies in chemistry–zoology at Pomona College and her M.Sc. (1977) and Ph.D. (1991) degrees at the University of British Columbia. Her M.Sc. dissertation work was on HbA_{1c} as a measure of diabetic control and her Ph.D. work on manganese deficiency effects in experimental diabetes. Her interest in vanadium as a therapeutic adjunct in diabetes began with a Nordic-Merrill Dow postdoctoral fellowship under John McNeill and has continued to the present, with the exception of a two-year appointment as research fellow in the Western Human Nutrition Research Center, Agricultural Research Service, USDA, San Francisco, where she worked on mathematical modeling of trace mineral metabolism. Since 1996, she has been a senior research associate in the Department of Chemistry at the University of British Columbia, in the Medicinal Inorganic Chemistry Group, under the direction of Chris Orvig.



John McNeill received his B.Sc. in pharmacy from the University of Alberta in 1960. He obtained his M.Sc. from the same institution two years later and his Ph.D. in Pharmacology at the University of Michigan in 1967. He became an assistant professor at Michigan State University in 1967 and then joined the Faculty of Pharmaceutical Sciences at the University of British Columbia in 1971. He served as Dean of this faculty from 1985 to 1995 and is the recipient of numerous awards for his research, including the Upjohn Award (Pharmacological Society of Canada), McNeil Award (Association of Faculties of Pharmacy of Canada), and Jacob Biely Award and Killam Award from the University of British Columbia. He is a founding Editor of the *Journal of Pharmacological and Toxicological Methods* and has published over 350 manuscripts, reviews, and book chapters. He has served on and chaired research committees for the Canadian Medical Research Council, Canadian Heart and Stroke Foundation, Canadian Diabetes Association, and the PMAC-Health Research Foundation. He currently serves on the jury of the Prix Galien Award.

Chris Orvig was born and raised in Montréal. He received his B.Sc. in chemistry from McGill University in 1976, and he subsequently pursued graduate studies (as an NSERC scholar) in Tc chemistry at M.I.T. with Alan Davison, receiving his Ph.D. in 1981. He was then an NSERC postdoctoral fellow with Ken Raymond at the University of California, Berkeley, in 1981–83. After one year with the late Colin Lock at McMaster University, he joined the University of British Columbia in 1984, where he is now Professor of Chemistry and Pharmaceutical Sciences and Director of the Medicinal Inorganic Chemistry Group. He is the inorganic chemistry editor of the *Canadian Journal of Chemistry* and he has received various research and teaching awards. He is currently (1999–2000) on sabbatical based in Münster, Germany, with F. E. Hahn and traveling the world delivering lectures.

The normal uptake and metabolism of glucose in nondiabetic individuals is initiated by a series of intracellular reactions known as the insulin signaling cascade.[13] Early in the insulin signaling cascade, insulin binds to the extracellular side of cell membranes at the insulin receptor sites,[14] initiating a series of phosphorylation/dephosphorylation steps, some of which are susceptible to substitute regulation by vanadium.[15] Absence of endogenously secreted insulin, or cellular resistance to the hormone, leads to inadequate disposal of blood glucose, the hallmark of diabetes mellitus. Because insulin is a protein, however, it is not orally active. As such, oral ingestion of exogenous insulin does not yield a biologically active hormone.[16] By contrast, vanadium compounds can be administered orally, thereby potentially eliminating or reducing significantly the need for daily insulin injections in diabetic individuals.

III. Oxidation States of V of Interest for Insulin Mimics

Vanadium is a group 5 transition metal with well-characterized complexes existing in oxidation states -3, -1, 0, $+1$, $+2$, $+3$, $+4$, and $+5$.[17] Under standard physiological conditions (pH 3−7, aerobic atmosphere, aqueous solution, ambient temperature), those oxidation states which are thermodynamically and kinetically possible are $+5$, $+4$, and $+3$,[2] the latter being associated mainly with specialized vanadocytes in certain marine life forms such as the tunicates *ascidia certodes* and *ascidia nigra*.[18] In simplified aqueous systems used to represent in vivo intracellular conditions, relevant vanadium species include vanadate (a mixture of $[HVO_4]^{2-}$ and $[H_2VO_4]^-$) and vanadyl, VO^{2+}.[19] In actual cell cultures and cell homogenates, vanadium, added either as V(V) or V(IV), rapidly forms complexes with glutathione, citrate, catecholamines, or proteins such as albumin and transferrin.[20] The intracellular fate of added V(III) salts has not been characterized.

Both V(V) and V(IV) inorganic salts have been extensively tested as insulin mimics, initially in vitro and subsequently in vivo, in a considerable variety of experimental models of diabetes. Sodium orthovanadate stimulates glucose uptake and glucose

oxidation in rat adipocytes, stimulates glycogen synthesis in rat diaphragm and liver, and inhibits hepatic gluconeogenesis, usually at millimolar concentrations of added vanadate or vanadyl.[11,21,22] Numerous studies have demonstrated insulin mimicking effects in a variety of tissues, with sodium orthovanadate [V(V)] usually being the modifier of choice.[23] Whether this insulin mimesis involves the same signaling pathways as insulin or an alternative route is not obvious and is still an unresolved question.[24−26]

A very exciting finding was that vanadate could be administered orally, with long-term insulin-like effects in vivo.[27] A series of in vivo studies demonstrated that oral vanadium(V) treatment of diabetic animals partially or completely restored liver and muscle enzyme activities involved in glycolysis, lipolysis, and glycogenesis,[28−30] without stimulating increased insulin synthesis.[31,32]

Since these early studies, a variety of strategies have been undertaken to improve the consistency and nontoxic efficacy of the insulin-mimetic response to vanadium as a therapeutic agent. Some improvement was found using inorganic V(IV) compounds[33] and even more with organic vanadium(IV) compounds.[6] Recent studies with chemically well-defined peroxovanadium(IV) and (V) compounds are intriguing due to the extraordinary in vitro potency demonstrated,[34] but their in vivo utility may be compromised by a lack of oral availability.[35]

IV. Design Factors for Effective Hormone Replacement by a Metal Complex

For metallocomplexes to be useful as biomimetic drugs, they must be able to cross biological membranes, generally by passive diffusion, because for most metal ions active or facilitated transport mechanisms are absent.[36,37] Biomimetic complexes should therefore have low molecular weight, neutral charge, and at least moderate stability. A high synthetic yield and known nontoxic metabolic products are also advantageous. The lipophilicity of the complex should be balanced with the hydrophilicity (i.e., water-solubility) of the molecule. Moreover, the metal−ligand complex should be thermodynamically and hydrolytically stable in water.[9]

Many pharmacologically useful metals, including vanadium, readily undergo hydrolysis in an aqueous environment.[38] This is especially true in a biological system if the formation constants of the metal complex are not high enough[38] and where the in vivo concentrations are usually low (micromolar to millimolar). The kinetics of complexation/decomplexation should also be considered. For insulin-mimetic compounds, delivery of the metal ion to key sites of insulin regulatory action, such as liver, adipose tissue, and/or skeletal muscle, requires slow kinetics of decomplexation to prevent rapid homeostatic removal of the vanadium as inorganic V(V) or V(IV).[39,40] In addition, from a biochemical point of view, in order for a metal complex to be useful in the treatment of type II diabetes, it must not induce increased insulin secretion but must instead serve as a (partial) hormone substitute or enhancing agent.[41] Ideally, it should also not exacerbate the already increased oxidative stress associated with diabetes of both types.[42]

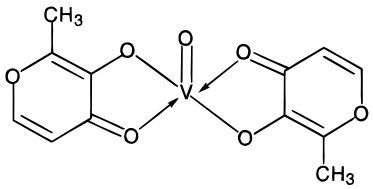
V. Structures and Physical Properties

Chemical compounds synthesized for insulin-mimetic studies will be discussed, first according to their chemistry and then their biological properties. Abbreviations are taken from the original publications.

A. BMOV

Bidentate ligands with one ionizable proton can be used to form neutral metal complexes (for instance, with vanadium). Oxygen-rich ligands (e.g., maltol) also tend to be water-soluble. These properties together (neutral charge and aqueous solubility) contribute to high oral bioavailability.[9,39] With these considerations in mind, a pentacoordinate, oxovanadium(IV) complex was developed, specifically as a potential insulin-mimetic agent.[43]

Bis(maltolato)oxovanadium(IV) (BMOV)[43] was synthesized by simple metathesis of vanadyl sulfate trihydrate and maltol (3-hydroxy-2-methyl-4-pyrone) (1:2). The ligand itself is commercially available and



BMOV, [VO(ma)$_2$]

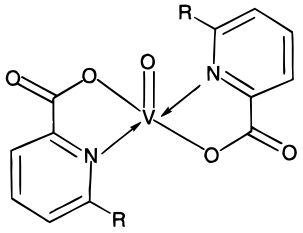
is an approved food additive in many countries, including Canada, the United Kingdom, and the United States. BMOV can be prepared in >90% yield in water, has a molecular weight of 317 and is soluble (millimolar scale) in a number of organic solvents as well as water.[44] Stability constants for the binding of one and two maltolato ligands to vanadyl are log $K_1 = 8.80$ and log $K_2 = 7.51$ and for the bis(ligand) complex log $\beta_2 = 16.31$.[44] The geometry around the vanadium in [VO(ma)$_2$] is square pyramidal.[44] BMOV has one unpaired electron, characteristic of the vanadyl unit, and a fairly high V=O stretching frequency in the infrared spectrum (995 cm^{-1}), suggesting that there is no ligand (or a weakly bound solvent) in the sixth position.

The redox chemistry of BMOV demonstrates an impressive lability in oxidation and reduction.[44] In ambient methanol or in any ambient alcoholic solvent, BMOV oxidizes to form an alkoxobis(maltolato)-oxovanadium(V) complex, *cis*-[VO(OR)(ma)$_2$], the oxidation kinetics being second order, a function of the concentrations of both the complex and molecular oxygen.[45,46] The reaction between BMOV and molecular oxygen, in a 4:1 ratio, gives the vanadium(V) species, consistent with the fact that BMOV undergoes a one-electron oxidation and O_2 is a four-electron oxidant. The observed rate constant is directly proportional to the molecular oxygen concentration, consistent with this stoichiometry and the overall

rate at 25 °C. Two pathways, aquo and hydroxo, give the dioxoanion, *cis*-$[VO_2(ma)_2]^-$ (which is not insulin-mimetic[47]) for oxidation of BMOV with O_2 in water.[46]

B.VPA and VO-MPA

Several V(IV) complexes with a $VO(N_2O_2)$ ligand coordination sphere have been proposed as insulin mimics. Examples are oxobis(picolinato)vanadium-(IV) (VPA) and bis(methylpicolinato)oxovanadium-(IV) (VO-MPA).[48,49]



VPA, R = H
VO-MPA, R = CH_3

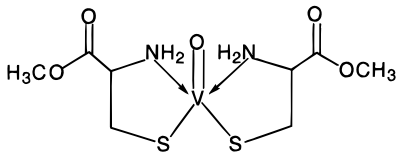
VPA is slightly soluble in water and, as an aqueous solution in an aerobic atmosphere, is susceptible to gradual oxidation. It is stable under inert gas in the solid state or when suspended in 5% acacia gum. The infrared absorption spectrum (KBr disk) included a V═O stretching frequency at 980 cm^{-1}.[50] The magnetic moment ($\mu_{eff} = 1.69$) supported a mononuclear vanadyl state, and EPR *g*-values and hyperfine coupling constants (*A* values) were consistent with oxovanadium(IV) complexation.[50]

VO-MPA has also recently been synthesized, characterized, and tested, both in vitro and in vivo, for insulin-mimetic activity.[48] For VO-MPA, the V═O stretching vibration appeared at 948 cm^{-1} and $\mu_{eff} = 1.96$; the EPR parameters were closely similar to those of VPA. On the other hand, the partition coefficient [P, *n*-octanol:buffer, pH 7.4 = 1.0, 6 h] for VO-MPA was nearly double that of VPA (0.595 vs 0.330).[50]

A recent extension of this type of vanadyl chelation (so far tested only in vitro) incorporates a number of different amino acids in a $VO(N_2O_2)$ coordination mode,[51,52] where the tetradentate N_2O_2 ligand is an ethylenediamine functionalized with two glycines or two methionines[51] or *N*-pyridylmethyl aspartate.[52] The latter was twice as effective as VOPA, both according to incorporation of 2-deoxyglucose in Erlich ascites tumor cells and by inhibition of free fatty acid release from rat adipocytes, but had a substantially lower partition coefficient (0.086).

C. VCME

A vanadyl bis(cysteine methyl ester) complex of vanadium(IV)[53] was synthesized by stirring a mixture of cysteine methyl ester hydrochloride and vanadyl sulfate (5:1) in 0.2 M borate buffer for 5−6 h, in air, at room temperature. The resultant purple solid product was found to be five coordinate around a central V(IV), with a square pyramidal geometry, and was relatively stable. Two strong IR (KBr disk) absorption bands at 956 and 945 cm^{-1} represented

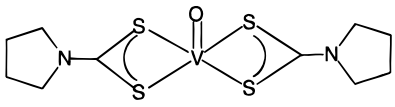


Vanadyl bis(cysteine methyl ester)

the V═O stretching frequencies, suggesting the presence of both cis and trans isomers.

D. V-P

V-P [oxobis(pyrrolidine-*N*-carbodithioato)vanadium-(IV)] was synthesized in >96% yield by combining ammonium pyrrolidine-*N*-carbodithioate with vanadyl sulfate (2:1) in ethanol.[54]

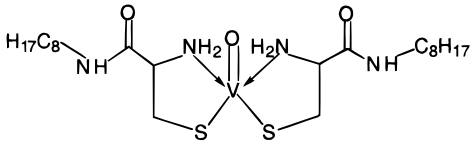


V-P

This complex has a VOS_4 coordination sphere, which had not been tried previously for insulin mimesis. In a series of V(IV) complexes, including V−O, V−N, and V−S coordination modes, which were tested for insulin-mimetic activity by inhibition of free fatty acid release in rat fat cell preparations, the V−P complex was the most effective.[55] The product was slightly soluble in a variety of organic solvents, including pyridine, dimethyl sulfoxide, and dimethylformamide, but insoluble in water, methanol, ethanol, and ether. It was also unstable in air. Solubilization for testing in vivo was possible using 5% acacia gum.

E. Naglivan

An N_2S_2-coordinated oxovanadium(IV) complex, naglivan [oxobis(*N*-octylcysteineamido)vanadium-(IV)], was synthesized in a two-step process.[56] From

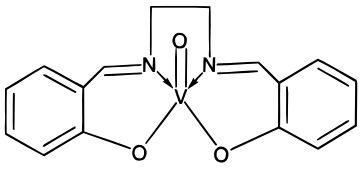


Naglivan

tert-butyloxycarbonylcysteine and octylamide as starting materials, cysteine di-*N*,*N*′-octylamide was produced, which was then combined (2:1) with vanadyl sulfate to yield (9.6%) a V(IV) complex. The complex was insoluble in water but could be administered as a suspension in 3% acacia gum by oral gavage for testing of insulin-mimetic potential.[56]

F. VO-SALEN

[*N*,*N*′-Disalicylidineethylenediamine]oxovanadium-(IV) (VO-SALEN) was prepared by mixing equimolar quantities of V(III) acetylacetonate and *N*,*N*′-disalicylideneethylenediamine (SALEN) as methanol solutions and stirring overnight in an open vessel.[57] The product was obtained in >95% yield and was char-

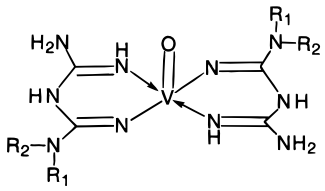


[*N,N'*-Disalicylidineethylenediamine] oxovanadium(IV), VO-SALEN

acterized by EPR, potentiometry, UV−vis, and IR ($\nu_{V=O}$ = 981 cm^{-1} (KBr disk), 953 cm^{-1} (DMSO)). The coordination geometry was octahedral in DMSO but square pyramidal in the solid. It was found to be stable, sparingly water-soluble, and orally effective in acute glucose-lowering.[58]

G. VO(metf)$_2$

VO(metf)$_2$·H$_2$O was prepared by combining an aqueous solution of vanadyl sulfate with an alkaline solution of metformin (1:2).[59] The formula weight was



Bis(biguanidato)oxo-vanadium(IV), VO(bg)$_2$

	R_1	R_2
big - biguanide	H	H
metf- metformin	CH_3	CH_3
Phenf - phenformin	H	$CH_2CH_2C_6C_5$

322, and the V=O stretching frequency (IR, KBr disk) was 929 cm^{-1}, low compared to other oxovanadium-(IV) complexes. The complex was air stable but hydrolytically unstable and insoluble in most organic solvents except DMSO.[59]

When deprotonated, the bidentate biguanides act as hard Lewis bases which bind readily to VO^{2+}, a hard Lewis acid. In general, biguanides have strongly basic primary dissociation constants and much weaker secondary ones. The conjugated double bond system is stabilized by intramolecular hydrogen bonding to form a six-membered ring. Metformin (Glucophage) is a common pharmaceutical used as an insulin-enhancing drug, either alone or in combination with other oral hypoglycemics, such as sulfonylureas.[59]

Coordination of the resonance-stabilized mono-deprotonated ligand is through the N-donor atoms, forming a rigid, planar, six-membered, π-delocalized chelate ring with the metal, thus enhancing overall thermodynamic stability. The probable (but unverified) coordination geometry is square pyramidal, with two biguanidato ligands in a trans arrangement around the base of the square pyramid and the V=O unit axial.[59]

VI. Biological Considerations

Before discussing the substantively different peroxovanadates and (nonoxo)vanadium(III) compounds, it is appropriate to summarize the biological considerations used to determine the relative pharmacological potency of proposed insulin-mimetic compounds. For in vitro testing, several enzyme systems have been used to demonstrate insulin mimesis. Phosphotyrosine phosphatases (PTPases) and tyrosine kinases play key regulatory roles in insulin receptor binding.[13] Early in the insulin signaling cascade, insulin, by binding on the extracellular side of cell membranes, activates the intracellular protein tyrosine phosphorylation of insulin receptors, which are membrane-spanning tyrosine-specific protein kinases.[14] PTPases and tyrosine kinases are sensitive to vanadium inhibition and stimulation, respectively,[60] and are sometimes used as markers of vanadium's insulin mimesis.[61]

In cell cultures of various types and in tissue homogenates, it is also possible to measure more directly the effect of exogenous vanadium on glucose and/or free fatty acid uptake, in comparison with added insulin.[62] These methods may be useful in initial screening, but they do not take into account oral availability, which can only be tested in vivo.

A. Animal Models for in Vivo Testing

For in vivo testing, a number of experimental models of diabetes in rodents have been used. Most widely accepted is the streptozotocin (STZ)-induced diabetic rat.[63] STZ is an antibiotic that specifically attacks the insulin-secreting β-cells in the pancreas in a dose-responsive fashion.[64] The STZ-diabetic rat model of diabetes is obtained by administering intravenous streptozotocin (STZ) to rats, usually at doses of 45−75 mg kg^{-1} of body weight. This results in a greatly reduced insulin secretory capacity of the rat pancreas and, hence, the development of diabetic characteristics (reduced insulin levels and elevated levels of glucose in blood and urine). STZ-treated rats are insulinopenic, hyperphagic, and catabolic. The model does not completely parallel type I diabetes in humans, in that STZ-diabetic rats can survive without administration of exogenous insulin (providing an untreated control group for comparison as an experimental benefit); however, it is relatively simple, inexpensive, reproducible, and reliable.

B. Evaluation of Potency by Glucose-Lowering

Moderate to good diabetic control, where diabetic control is generally defined as glucose lowering to less than 9 mM in plasma, has been obtained in the streptozotocin (STZ)-diabetic rat with V(V) or V(IV) at oral doses of between 0.1 and 0.7 mmol kg^{-1} day^{-1}.[65,66]

With BMOV, it has been possible to achieve effective blood glucose lowering at an initial dose of 0.4 mmol kg^{-1} day^{-1}, which is decreased to 0.2 mmol kg^{-1} day^{-1} for maintenance, with no evidence of toxicity over a six month period of administration in STZ-diabetic rats.[67,68] This oral administration was in the drinking water, which allows for a prolonged period of oral intake. A similar dose and mode of administration for VPA (in the drinking water, 0.75 mg mL^{-1}, approximately 9 mg V day^{-1})[49] led to 22% glucose-lowering (compared to 50%) for BMOV.[43] VPA, when

administered by oral gavage initially at the lower dose (2.2−2.5 mg V day^{-1}, equivalent to approximately 10 mg V kg^{-1} day^{-1} or 0.2 mmol kg^{-1} day^{-1}), led to normalization of blood glucose levels in diabetic rats within 7 days; the rats remained nearly normoglycemic for an additional 30 days with no treatment.[48] This oral administration (gavage) indicates a once-a-day introduction directly into the stomach, which would likely override any homeostatic mechanisms limiting gastrointestinal absorption. Faster glucose-lowering could be achieved by increasing the dose (5.3−6.2 mg V day^{-1} by oral gavage) but at the expense of undesirable side effects, principally diarrhea.[50]

The 10 mg V kg^{-1} day^{-1} test dose in STZ-diabetic rats has been the assay of choice for a number of vanadium-containing insulin-mimetic complexes. VO-MPA, 10 mgV kg^{-1} day^{-1}, given orally by gavage as a 5% acacia gum suspension, led to sustained glucose-lowering over an 80 day period following cessation of treatment; the decreased body weight gain and increased bilirubin levels, seen in the early stages of treatment, were correctable by lowering the dose to 5 mg V kg^{-1} day^{-1}.[48] V−P was administered *per os* at 10 mg V kg^{-1} day^{-1} to STZ-diabetic rats for 2 days to achieve normoglycemia, followed by a maintenance dose of 5 mg V kg^{-1} day^{-1} (0.1 mmol kg^{-1} day^{-1}). Intraperitoneal (i.p.) administration of this compound proved to be more effective than oral treatment, but both VO-MPA and V−P achieved significant glucose-lowering.[55] At this same dose, VCME in 5% acacia gum given by oral gavage was slightly more effective in normalizing plasma glucose levels (within 24 h) than other vanadyl complexes (ligands such as malonate, oxalate, salicylaldehyde, and tartrate) tested in parallel with VCME.[69] There was no obvious toxicity at this dose; however, at 10 times the glucose-lowering dose, all the test animals died of diarrhea within 4 days.[70]

Naglivan, at doses of 5−15 mg V kg^{-1} day^{-1} (0.1−0.3 mmol kg^{-1} day^{-1}, by oral gavage) effectively lowered blood glucose levels to near normal in STZ-diabetic rats; however, the onset of action was significantly slower than that seen with inorganic vanadium compounds used in parallel.[56,71] No obvious toxicity derived from naglivan at the doses administered.

$VO(metf)_2 \cdot H_2O$, 0.12 mmol kg^{-1}, suspended in 3% acacia gum, was administered i.p. to five STZ-diabetic rats with significant glucose-lowering effect. Observed toxic effects (pronounced diarrhea) may have been due to the high pH (11.5) of the preparation.[59] Oral gavage testing in a separate treatment group, at a dose of 0.60 mmol kg^{-1}, resulted in six out of six rats responding with normalized plasma glucose levels, within 24 h; however, the response was not sustained past that time point. Metformin alone at the same dose had no effect. $VO(metf)_2$ has only been tested acutely so far, both by gavage and i.p. administration over a period of 72 h, but for this limited time frame, the glucose-lowering capacities were similar to BMOV.[59]

Glucose-lowering potency of BMOV given i.p. at a dose of 1.275 mg V kg^{-1} has also been very recently compared with that of vanadyl sulfate, vanadyl acetylacetonate [$VO(acac)_2$] (commercially available), and a close analogue vanadyl ethylacetylacetonate [VO-EtAc].[72] At this dose, only $VO(acac)_2$ exhibited any glucose-lowering effect in STZ-diabetic rats; however, this compound is more air sensitive than BMOV. Comparison of these same four compounds administered as dissolved salts in drinking water (approximately 0.4 mmol kg^{-1} day^{-1}) showed roughly the same potency for all the organically chelated vanadyl compounds (all more effective than vanadyl sulfate); however, an apparent taste aversion led to a lesser daily vanadium intake for $VO(acac)_2$ compared to BMOV.[72]

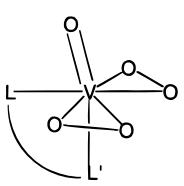
Blood glucose levels in alloxan-induced diabetic rats decreased from hyperglycemic to hypoglycemic during oral intubation with 7.5 mg V kg^{-1} day^{-1} as VO−SALEN for a period of 30 days. Withdrawal of treatment brought an immediate reversion to hyperglycemia.[58]

Other animal models of diabetes, in specially developed rodent strains that are spontaneously diabetic, include the BioBreeding (BB) Wistar rat, a spontaneous model of diabetes that closely resembles type I diabetes,[73] the *fa/fa* Zucker rat, a model of obesity and mild glucose intolerance,[74] *ob/ob* and *db/db* mice that are obese and glucose intolerant,[75] and a variety of other specialized strains.[63] The key end point of interest is usually blood glucose-lowering, which is known to be correlated closely with plasma lipid-lowering in diabetic animals.

VII. Peroxovanadates

A. $[VO(O_2)_2(L\text{-}L')]^{n-}$

Two of the earliest discrete diperoxovanadate(V) compounds, potassium oxodiperoxo(pyridine-2-carboxylato)vanadate(V) and potassium oxodiperoxo(3-hydroxypyridine-2-carboxylato)vanadate(V),[76] were direct descendants of analogous chromium complexes,[77,78] which had been shown to improve membrane fluidity and increase the rate of insulin uptake.[78] Although these peroxovanadates are stable indefinitely in the solid state, they are prone to decomposition in aqueous solutions. Both have a distorted bipyramidal ligand geometry around the vanadium ion[76] and have been shown to be effective in stimulating insulin receptor kinase (IRK) activity in hepatoma cells and inhibiting phosphotyrosine phosphatase (PTPase) activity in rat liver endosomes.[34] In the latter study, 12 different bisperoxovanadium compounds of the $[VO(O_2)_2L-L']^{n-}$ type[34]



$[VO(O_2)_2(L–L')]^{n-}$ n=1-3

Ligandoxobis(peroxo)vanadate(V)

were compared. The most stable, potassium oxodiperoxo(1,10-phenanthroline)vanadate(V) trihydrate

[bpV(phen)], is heptacoordinate, with the geometry about the V atom being pentagonal bipyramidal.[79] The planes formed by the two peroxo groups and the V atom were found to be bent toward each other with a dihedral angle of 22.1(3)°, approximately perpendicular to the phenanthroline ligand. The oxo ligand lies in the plane of the vanadium−phenanthroline moiety, trans to the second phenanthroline N atom.

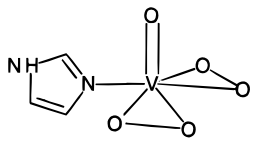
BpV(phen) was tested in vivo in fasted female Sprague−Dawley rats, by intrajugular injection of 6 μmol kg^{-1}, a dose which resulted in glucose-lowering equivalent to 15 μg kg^{-1} of insulin administered by the same method. At doses of 0.75−6 μmol kg^{-1}, bpV(pic), bpV(phen), and bpV(Me$_2$phen) were effective in lowering plasma glucose in BB rats whether given i.v., i.p., or subcutaneously.[80] Only bpV(phen), 20−200 μmol kg^{-1}, was shown to be effective when administered by oral gavage.[35] Demonstrably increased insulin binding to intact rat adipocytes[81] (but not human NIDDM fat cells[82]) in the presence of 0.5 mM bpV(pic), possibly due to increased insulin receptor affinity, suggests an insulin-sparing mechanism of action for peroxovanadates of this type.[82]

Effective concentrations of all the peroxovanadates tested for in vitro inhibition of phosphotyrosine phosphatase (PTPase) and stimulation of insulin receptor tyrosine kinase were in the 5−80 mM vanadium range.[34] The nature of the ancillary ligand appeared to have profound effects on the specificity and potency of the complex, with increasing bulkiness of the phenolate ligand, whether polar [e.g., carboxylation in bpV(bipy)] or nonpolar [e.g., methylation in bpV(phen)], decreasing IRK stimulation, and increasing methylation of the phenanthroline ligand reducing PTPase inhibition.[34]

Synthesis and characterization of a large number of peroxovanadate heteroligands, both mono- and polydentate,[83] revealed a range of stabilities toward decomposition in aqueous solution, depending on the nature of the heteroligand. The fact that none of the peroxovanadates formed can be considered hydrolytically stable and all are subject to redox processes which ultimately result in radical formation with the potential for increased intracellular oxidative stress[84] may limit biomedical utility.[83]

B. [VO(O$_2$)$_2$(him)]−

A six-coordinate bisperoxovanadium(V) imidazole compound was synthesized in 70% yield, by combining vanadium pentoxide dissolved in 30% hydrogen peroxide with imidazole (1:4). The vanadium was six-coordinate (five V−O bonds and one V−N), in a pentagonal pyramid, with the oxo group in an axial position.[85]



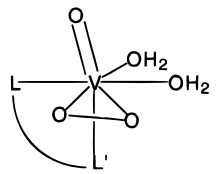
[VO(O$_2$)$_2$(Him)]$^-$

Imidazoleoxobisperoxovanadate(V)

An aqueous solution of the complex enhanced insulin receptor autophosphorylation in human liver cell culture, as well as increased glucose transport in rat adipocytes, at concentrations ranging from 1 μM to 1 mM.[85] The coordination of vanadium(V) to imidazole presents structural analogies to the coordination of vanadium to histidine residues in vanadium-containing haloperoxidases[86] and some phosphorylases.[87]

C. [VO(O$_2$)(H$_2$O)$_2$(L-L′)]^n−

A variety of well-characterized monoperoxovanadate(V) complexes have also recently been prepared and tested in vitro and by intraperitoneal and/or subcutaneous injection in vivo.[80,88] Oxoperoxopicolinatovanadium(V) dihydrate [mpV(pic)] was effective in achieving a 20% decrease in plasma glucose in STZ-diabetic Sprague Dawley and insulin-treated (0.5−2.0 U day^{-1}) BB rats at a lowest effective dose (LED) of 0.4 μmol kg^{-1}, while the lowest dose producing mortality was more than 15 times higher. By contrast, pyridine-2,6-dicarboxylatooxoperoxovanadate monohydrate [mpV(2,6-pdc)]$^-$ had an LED of 24 μmol kg^{-1}, which was twice the lowest dose producing mortality.[80] In each case, O- or N-donor atoms were bound to two or more sites in the V(V) trigonal bipyramidal coordination sphere, usually with a −1 or −2 charge.[80] Bis(oxalato)oxoperoxovanadate [mpV(ox)$_2$]$^{3-}$ was only minimally stimulatory on [^{14}C]glucose incorporation into diaphragm glycogen, unlike mpV(pic) which was 9 times greater than control levels.[88]

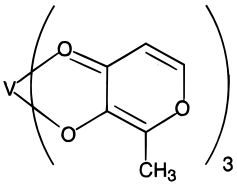


[VO(O$_2$)(H$_2$O)$_2$(L–L')]$^{n-}$ n=0,1

Ligandoxoperoxovanadium(V)

VIII. V(III) Complexes

Synthesis of hexacoordinated vanadium(III) pyrone and pyridinone complexes produced stable, lipophilic complexes which were water soluble.[89]



Tris(maltolato)vanadium(III), [V(ma)$_3$]

Aqueous solutions of these compounds are governed by complex equilibria for protonated and deprotonated ligands, depending on pH and concentrations of vanadium and ligands. Biological testing, both i.p. and by oral gavage, demonstrated the glucose-lowering capability of several V(III)-containing complexes, with V(ma)$_3$ proving to be the most

potent by these standards. Although no attempt has yet been made to establish the speciation in vivo, it is presumed that at physiological pH, V(III) complexes would oxidize rapidly to V(IV) or V(V).

IX. Vanadium as a Therapeutic Agent

A. Human Trials

Recently, limited clinical trials of vanadium compounds have been initiated on human type I (IDDM) and type II (NIDDM) diabetic subjects. In diabetes, glucose uptake into peripheral tissues such as skeletal muscle and fat is impaired and glucose utilization in the energy-dependent processes within cells is abnormal.[16] Diabetes may be a consequence of defective insulin binding, resulting in poor intracellular regulation of energy metabolism.[13] Diabetic individuals are generally characterized as either type I, in which insulin production is deficient (IDDM), or type II, later onset, non-insulin dependent (NIDDM), in which a normal or even excessive amount of insulin production fails to result in well-tuned intracellular signaling.[90]

Treatment of generally healthy subject recruits with sodium orthovanadate (125 mg day^{-1}) for 2 weeks resulted in significant increases in mean rates of glucose metabolism in two out of five subjects with IDDM and in five out of five subjects with NIDDM.[91] There were decreased insulin requirements in the IDDM subjects and lowered serum cholesterol levels in all subjects. Treatment of NIDDM subjects with vanadyl sulfate (100 mg day^{-1}) for 3 weeks caused an improved insulin sensitivity, a reduction in hepatic glucose production, and an increased rate of glucose disposal, all of which were sustained for 2 weeks after treatment was withdrawn.[92] In both of the studies, there were reported incidences of mild gastrointestinal intolerance.

Vanadyl therapy for 6 weeks at 100 mg day^{-1} of $VOSO_4 \cdot 3H_2O$ resulted in improved insulin sensitivity in three of five human NIDDM subjects (who were all also being treated with oral hypoglycemics during the study)[93,94] while basal hepatic glucose production was unchanged. In a more recent study,[95] also with $VOSO_4 \cdot 3H_2O$, 25 mg V day^{-1}, there was no change in glucose and lipid metabolic parameters and the peak serum vanadium level was 16.0 ± 5.3 $\mu g\ L^{-1}$. By comparison, doubling the intake of vanadyl sulfate (to 50 mg V day^{-1}) resulted in 5 times higher peak serum vanadium (82.4 ± 43.2 $\mu g\ L^{-1}$) and improved insulin sensitivity but still no significant change in plasma glucose levels.[95] There was no increase in thiobarbituric acid reactive substances (an indicator of in vivo lipid peroxidation) at these doses (Goldfine, personal communication).

B. Desirable Features

For appreciable biological utility, proposed insulin replacement metal complexes should have oral activity, a reasonable window of optimal pharmacological effect, tissue uptake preferentially into normally insulin-rich tissues, e.g., liver, muscle, and pancreas, a consistent pattern of absorption and retention, and a low dose requirement.

Few vanadium-containing insulin-mimetic complexes have been evaluated for pattern of tissue uptake. Those that have include BMOV[39] and VO-MPA.[50] For BMOV, both oral and intraperitoneal (i.p.) administration of ^{48}V-BMOV in a carrier-added form were compared with carrier-added ^{48}V-$VOSO_4$.[39] From these studies, the absorption of vanadium from an oral dose of ^{48}V-BMOV was determined to be about twice as high as that from ^{48}V-vanadyl sulfate; and tissue localization resulting from administration of the two compounds differed. Compartmental analysis of the results showed that the proportion of vanadium taken up by liver following BMOV treatment was almost 4 times higher than with $VOSO_4$ treatment, whereas that taken up by kidney was less than 50% higher, and that by bone (at 24 h) was almost 3 times higher. The ratios of vanadium contents predicted by the model in bone:kidney:liver was 8:3:2 for BMOV and 6:4:1 for $VOSO_4$. The average increase in uptake into liver, kidney, and bone was 2.7 times higher in BMOV compared to $VOSO_4$, correlating well with the increased potency of BMOV over $VOSO_4$ observed previously.[67]

By contrast, tissue uptake resulting from oral administration of VO-MPA in bone:kidney:liver was roughly 7:6:1 and from i.p administration was substantially different (approximately 11:3.3:1). For VO−PA administered i.p., accumulation in bone relative to kidney and liver was 35:4.7:1.[50]

C. Potential Drawbacks

The toxicology of vanadium has been reviewed recently.[96] Vanadium's toxicity varies by route of administration, by species tested, and, by analogy with other metals, according to the complexation of the metal.[97,98] Principal signs of vanadium toxicity are gastrointestinal distress[92,99,100] and, more rarely, green tongue.[100] Vanadium accumulation in tissues (especially bone) with long-term therapeutic use is a concern; however, there is no evidence to date that increased storage in bone is harmful.[101] Further studies elucidating residence times and clearance rates of vanadium compounds from specific tissues[39] are needed in order to assess this potential problem.[102]

X. Comparison with Other Metal-Containing Insulin Mimics

A. Molybdenum and Tungsten Analogues

In common with vanadium, molybdenum and tungsten also form stable oxoanions, which undergo complex hydrolysis−polymerization reactions in aqueous solutions, especially at low pH.[103] Vanadium(V), Mo(VI), and W(VI) all have the same electronic configuration (d^0) and favor octahedral coordination in metallocomplexes. Like vanadium, molybdenum and tungsten can exist in multiple oxidation states under physiological conditions[104] and can potentially couple oxide or proton transfer with electron transfer when complexed appropriately.[105,106] As exogenously added inorganic salts, all have been shown to have similar effects on liver enzyme activities in the

glycolytic pathway,[107] although the comparative potency observed for glucose metabolism vanadate > tungstate > molybdate was the same as for relative inhibition of alkaline phosphatase activity.[107] The in vitro effects could be substantially strengthened by mixing either sodium molybdate or sodium tungstate with hydrogen peroxide just prior to addition to isolated cell suspensions,[108] a potentiating effect previously seen with vanadate.[109,110] An obvious next step was to form permolybdates and pertungstates, analogous to pervanadates (vide supra), which could then be compared for insulin mimesis in isolated cell assays.[111]

Oxodiperoxo complexes of Mo(VI) and W(VI) are particularly efficient oxygen-atom transfer and one-electron oxidizing agents, with a broad pH range of effectiveness in aqueous solution (at least pH 0−7).[112] Pervanadates were about 2 orders of magnitude more potent than pMo and pW in PTPase inhibition; however, the greater hydrolytic stability and lower toxicity of molybdates and tungstates compared to vanadates has encouraged continued development of these potentially interesting insulin mimics, as evidenced by a recent patent application.[113] In vivo oral administration of ammonium pertungstate (1 mg mL^{-1} day^{-1}) for 2 weeks to STZ-diabetic rats resulted in normalization of blood glucose and of liver hexose, hexosamine, and sialic acid contents.[114] Insulin levels were not reported. A hexacoordinate *cis*-bis(maltolato)bisoxomolybdenum(VI) has been synthesized and characterized but not biologically evaluated (Orvig, personal communication). The fact that molybdenum is readily absorbed is a definite advantage for more accurate dose determination.[115] A possible disadvantage is that molybdates and tungstates are less potent than vanadates in all systems tested so far.[111] Experimentation with a variety of coordination complexes for optimal insulin mimesis seems likely to follow.

B. Zinc Insulin Mimesis

Zinc coordination and redox properties are entirely unlike those of vanadium, yet some studies have revealed insulin-like glucose-lowering and other antidiabetic effects.[116] There is some question whether this is more a pharmacological or a nutritional effect.[117] Zinc, as a divalent cation, has a completely filled d^{10} electronic configuration and has no ligand-field stabilization energy for any particular coordination geometry,[118] tetrahedral being therefore favored. Ligand binding of Zn−N, Zn−O, and Zn−S are all possible, and Zn easily undergoes relatively rapid ligand exchange. Zn is not redox active, and only the divalent oxidation state is thermodynamically possible under physiological conditions. Nonetheless, zinc appears to play an essential role in maintenance of glucose metabolism, and simple oral supplementation has been associated with positive symptomatic improvements.[119,120]

C. Combination with Lithium and Other Metals

Other metal ions which have shown some promise as insulin mimetics include lithium, magnesium, and chromium, each for different reasons.[119] For lithium, the most promising in vivo effects have been in combination with vanadium;[121] for magnesium, chronic supplementation in type II diabetes has given the best pharmacological effects;[122,123] while for chromium, the effects are generally considered to be closely tied to marginal chromium nutritional status and are therefore related to recovery from deficiency.[124] The most intriguing feature of lithium's effects is the potential for synergistic glucose and plasma lipid lowering in concert with vanadium.[121,125] Combining 0.3 g L^{-1} lithium carbonate with 0.05 g L^{-1} sodium orthovanadate (1/10 the usual dose) in the drinking water of diabetic rats normalized plasma glucose (with no change in plasma insulin) and restored tissue antioxidant enzymes to normal levels of activity.[121]

XI. Future Studies and Concluding Remarks

As more and more of the intricacies of the insulin regulatory cascade are elucidated, we can look forward to a more narrowly defined locus of vanadium's insulin mimesis. This in turn will permit fine-tuning of an appropriate ligand environment for vanadium coordination complexes that are optimally configured for insulin-mimetic activity in vivo. As more is known about the coordination chemistry of V(III), V(IV), and V(V), we can also anticipate gaining greater a priori control over tissue targeting, such that cellular uptake of vanadium is preferentially directed toward those tissues which normally require insulin for full metabolic function. These tissues include, most notably, liver, skeletal muscle, and adipose tissue. Attention to the chemical and physical requirements for effective hormone replacement may yield molecular entities that are more readily absorbed than the notoriously poorly absorbed inorganic vanadium salts, with the result that dose determinations will be more precise and potential for toxicity and inadvertent organ vanadium accumulation lessened.

The most pressing needs currently for improving the pharmacological utility of vanadium-containing compounds are in the areas of comparative tissue uptake, distribution and excretion for a variety of vanadium coordination complexes, and closer definition of the metabolic fate of these complexes. As has already been done for selenium-containing compounds,[97] we can anticipate soon that oxovanadium(IV), peroxovanadate(V), and vanadium(III) complexes will be defined with regard to their point of entry into in vivo metabolic pathways. This information, in conjunction with more detailed knowledge of the physiological and biochemical changes accompanying diabetic symptomatology, will lead to more focused efforts in the development of vanadium-containing insulin-mimetic compounds. At the very least, further research into the mechanism of action of these complexes will be of value until directed gene therapy can obviate the need for diabetic symptom relief, which is currently by necessity only a stopgap approach.

In conclusion, the desirable features of vanadium-containing insulin-mimetic agents, including oral activity and potential for targeted tissue uptake, tend

to outweigh the potential drawbacks, e.g., tissue accumulation and uncertainty of initial dose determination, such that, for the foreseeable near future (10−20 years), these compounds present an opportunity for substantial advance over available diabetic therapies. In addition, they provide unprecedented and exciting avenues of investigation in the mechanism of insulin action and course of development of diabetic symptomatology. Whether alone or in combination with other metals, vanadium-containing anti-diabetic agents are set to revolutionize the way we look at diabetes.

XII. Acknowledgments

The authors gratefully acknowledge the Medical Research Council of Canada for grant support, the Natural Sciences and Engineering Research Council of Canada for personnel support and scholarships, and our many talented co-workers, who are listed in the references. We would also like to thank Nycomed Salutar, Kinetek Pharmaceuticals, Inc., and Angiotech Pharmaceuticals, Inc., for research support.

XIII. Abbreviations

BMOV	bis(maltolato)oxovanadium(IV)
VPA	oxobis(picolinato)oxovanadium(IV)
VO-MPA	bis(methylpicolinato)oxovanadium(IV)
VCME	vanadyl bis(cysteine methyl ester)
V-P	oxobis(pyrrolidine-*N*-carbodithioato)oxovanadium(IV)
Naglivan	oxobis(*N*-octylcysteineamido)vanadium(IV)
VO−SALEN	[*N*,*N*′-disalicylidineethylenediamine]oxovanadium(IV)
$VO(metf)_2$	bis(*N*,*N*′-dimethylbiguanidato)oxovanadium(IV)
$VO(bg)_2$	bis(biguanidato)oxovanadium(IV)
PTPase	phosphotyrosine phosphatase
STZ	streptozotocin
bpV(phen)	oxodiperoxo(1,10-phenanthroline)vanadate(V)
bpV(bipy)	oxodiperoxo(bipyridine)vanadate(V)
mpV(pic)	oxoperoxopicolinatovanadium(V)
IDDM	insulin-dependent diabetes mellitus
NIDDM	noninsulin-dependent diabetes mellitus
i.v.	intravenous
i.p.	intraperitoneal

XIV. References

(1) Djordjevic, C.; Puryear, P. C.; Vuletic, N.; Allelt, C. J.; Sheffield, S. J. *Inorg. Chem.* **1988**, *27*, 2926.
(2) Chasteen, N. D.; Grady, J. K.; Holloway, C. E. *Inorg. Chem.* **1986**, *25*, 2754.
(3) Butler, A.; Walker, J. V. *Chem. Rev.* **1993**, *93*, 1937.
(4) Barnett, P.; Hemrika, W.; Dekker, H. L.; Muijsers, A. O.; Renirie, R.; Wever, R. *J. Biol. Chem.* **1998**, *273*, 23381.
(5) Nielsen, F. H. In *Metal Ions in Biological Systems: Vanadium and Its Role in Life*; Sigel, H., Sigel, A., Eds.; Marcel Dekker: New York, 1995; Vol. 31, p 543.
(6) Orvig, C.; Thompson, K. H.; Battell, M.; McNeill, J. H. *Metal Ions in Biological Systems: Vanadium and Its Role in Life*; Sigel, H., Sigel, A., Eds.; Marcel Dekker: New York, 1995, Vol. 31, p 575.
(7) Fantus, I. G.; Tsiani, E. *Mol. Cell. Biochem.* **1998**, *182*, 109.
(8) Thompson, K. H. *BioFactors*, in press.
(9) Thompson, K. H.; McNeill, J. H.; Orvig, C. In *Topics in Biological Inorganic Chemistry*; Clarke, M. J., Sadler, P. J., Eds.; Springer-Verlag: Heidelberg, 1999, Vol. 2, p 139.
(10) Czech, M. P. *Annu. Rev. Biochem.* **1977**, *46*, 359.
(11) Tolman, E. L.; Barris, E.; Burns, M.; Pansisni, A.; Partridge, R. *Life Sci.* **1979**, *25*, 1159.
(12) Bosch, F.; Arino, J.; Gomez-Foix, A. M.; Giunovart, J. J. *J. Biol. Chem.* **1987**, *262*, 218.
(13) Kahn, C. R.; White, M. F. *J. Clin. Invest.* **1988**, *82*, 1151.
(14) White, M. F.; Shaleson, S. E.; Keutmann, H.; Kahn, C. R. *J. Biol. Chem.* **1988**, *263*, 2969.
(15) Cheatham, B.; Kahn, C. R. *Endocr. Rev.* **1995**, *16*, 117.
(16) Lienhard, G. E.; Slot, J. W.; James, D. E.; Mueckler, M. M. *Sci. Am.* **1992**, *267*, 86.
(17) Butler, A.; Carrano, C. J. *Coord. Chem. Rev.* **1991**, *109*, 61.
(18) Butler, A. In *Vanadium in Biological Systems*; Chasteen, N. D., Ed.; Kluwer Academic Publishers: Dordrecht, 1990; p 25.
(19) Willsky, G. R. In *Vanadium in Biological Systems*; Chasteen, N. D., Ed.; Kluwer Academic Publishers: Dordrecht, 1990; p 1.
(20) Chasteen, N. D.; Lord, E. M.; Thompson, H. J.; Grady, J. K. *Biochim. Biophys. Acta* **1986**, *884*, 84.
(21) Shechter, Y.; Karlish, S. J. D. *Nature (London)* **1980**, *284*, 556.
(22) Dubyak, G. R.; Kleinzeller, A. *J. Biol. Chem.* **1980**, *255*, 5306.
(23) Shechter, Y. *Diabetes* **1990**, *39*, 1.
(24) Elberg, G.; He, Z.; Li, J.; Sekar, N.; Shecther, Y. *Diabetes* **1997**, *46*, 1684.
(25) Li, J.; Elberg, G.; Crans, D. C.; Shechter, Y. *Biochemistry* **1996**, *35*, 8314.
(26) Tsiani, E.; Fantus, I. G. *Trends Endocrinol. Metab.* **1997**, *8*, 51.
(27) Heyliger, C. E.; Tahiliani, A. G.; McNeill, J. H. *Science* **1985**, *227*, 1474.
(28) Gil, J.; Miralpeix, M.; Carreras, J.; Bartrons, R. *J. Biol. Chem.* **1988**, *263*, 1868.
(29) Blondel, O.; Simon, J.; Chevalier, B.; Portha, B. *Am. J. Physiol.* **1990**, *258*, E459.
(30) Brichard, S. M.; Assimacopoulos-Jeannet, F.; Jeanrenaud, B. *Endocrinology* **1992**, *131*, 311.
(31) Bendayan, M.; Gingras, D. *Diabetologia* **1989**, *32*, 561.
(32) Ramanadham, S.; Cros, G. H.; Mongold, J. J.; Serrano, J. J.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **1990**, *68*, 486.
(33) Dai, S.; Thompson, K. H.; Vera, E.; McNeill, J. H. *Pharmacol. Toxicol.* **1994**, *75*, 265.
(34) Posner, B. I.; Faure, R.; Burgess, J. W.; Bevan, A. P.; Lachance, D.; Zhang-Sun, G.; Fantus, I. G.; Ng, J. B.; Hall, D. A.; Soo Lum, B.; Shaver, A. *J. Biol. Chem.* **1994**, *269*, 4596.
(35) Yale, J.-F.; Vigeant, C.; Nardolillo, C.; Chu, Q.; Yu, J.-Z.; Shaver, A.; Posner, B. I. *Mol. Cell. Biochem.* **1995**, *153*, 181.
(36) Dreosti, I. E. *Nutrition* **1993**, *9*, 542.
(37) Fairweather-Tait, S. J.; Minihane, A.-M.; Eagles, J.; Owen, L.; Crews, H. M. *Am. J. Clin. Nutr.* **1997**, *65*, 970.
(38) Clevette, D. J.; Orvig, C. *Polyhedron* **1990**, *9*, 151.
(39) Setyawati, I. A.; Thompson, K. H.; Sun, Y.; Lyster, D. M.; Vo, C.; Yuen, V. G.; Battell, M.; McNeill, J. H.; Ruth, T. J.; Zeisler, S.; Orvig, C. *J. Appl. Physiol.* **1998**, *84*, 569.
(40) Thompson, K. H.; McNeill, J. H.; Orvig, C. *Rev. Port. Quim.* **1997**, *4*, 3−10.
(41) Reaven, G. M. *Annu. Rev. Med.* **1993**, *44*, 121.
(42) Wolff, S. P.; Jiang, Z. Y.; Hunt, J. V. *Free Radical Biol. Med.* **1991**, *10*, 339.
(43) McNeill, J. H.; Yuen, V. G.; Hoveyda, H. R.; Orvig, C. *J. Med. Chem.* **1992**, *35*, 1489.
(44) Caravan, P.; Gelmini, L.; Glover, N.; Herring, F. G.; Li, H.; McNeill, J. H.; Rettig, S. J.; Setyawati, I. A.; Shuter, E.; Sun, Y.; Tracey, A. S.; Yuen, V. G.; Orvig, C. *J. Am. Chem. Soc.* **1995**, *117*, 12759.
(45) Hanson, G. R.; Sun, Y.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 6507.
(46) Sun, Y.; James, B. R.; Rettig, S. J.; Orvig, C. *Inorg. Chem.* **1996**, *35*, 1667.
(47) Yuen, V. G.; Caravan, P.; Gelmini, L.; Glover, N.; McNeill, J. H.; Setyawati, I. A.; Zhou, Y.; Orvig, C. *J. Inorg. Biochem.* **1997**, *68*, 109.
(48) Sakurai, H.; Fujii, K.; Watanabe, H.; Tamura, H. *Biochem. Biophys. Res. Commun.* **1995**, *214*, 1095.
(49) Melchior, M.; Thompson, K. H.; Jong, J. J.; Rettig, S. J.; Shuter, E.; Yuen, V.; Zhou, Y.; McNeill, J. H.; Orvig, C. *Inorg. Chem.* **1999**, *38*, 2288.
(50) Fujimoto, S.; Fujii, K.; Yasui, H.; Matsushita, R.; Takada, J.; Sakurai, H. *J. Clin. Biochem. Nutr.* **1997**, *23*, 113.
(51) Kawabe, K.; Tadokoro, M.; Kojima, Y.; Fujisawa, Y.; Sakurai, H. *Chem. Lett.* **1998**, *1998*, 9.
(52) Tawa, R.; Uchida, K.; Taniyama, J.; Fujisawa, Y.; Fujimoto, S.; Nagaoka, T.; Kanamori, K.; Sakurai, H. *J. Pharm. Pharmacol.* **1999**, *51*, 119.
(53) Sakurai, H.; Hamada, Y.; Shimomura, S.; Yamashita, S.; Ishizu, K. *Inorg. Chim. Acta* **1980**, *46*, L119.
(54) McCormick, B. J. *Inorg. Chem.* **1968**, *7*, 1965.
(55) Watanabe, H.; Nakai, M.; Komazawa, K.; Sakurai, H. *J. Med. Chem.* **1994**, *37*, 876.
(56) Cam, M. C.; Cros, G. H.; Serrano, J.-J.; Lazaro, R.; McNeill, J. H. *Diab. Res. Clin. Pract.* **1993**, *20*, 111.
(57) Bonadies, J. A.; Carrano, C. J. *J. Am. Chem. Soc.* **1986**, *108*, 4088.
(58) Durai, N.; Saminathan, G. *J. Clin. Biochem. Nutr.* **1997**, *22*, 31.
(59) Woo, L.; Yuen, V. G.; Thompson, K. H.; McNeill, J. H.; Orvig, C. Submitted for publication.

(60) Gordon, J. A. *Methods Enzymol.* **1991**, *201*, 477.
(61) Shechter, Y.; Meyerovitch, J.; Gefel, D.; Bruck, R.; Shisheva, A.; Elberg, G.; Libman, J.; Shanzer, A. *Front. Endocrinol.* **1993**, *1*, 51.
(62) Fantus, I. G.; Ahmad, F.; Deragon, G. *Diabetes* **1994**, *43*, 375.
(63) Bell, R. H. J.; Hye, R. J. *J. Surg. Res.* **1983**, *35*, 433.
(64) Rakieten, N. *Cancer Chemother. Rep.* **1963**, *29*, 91.
(65) Mongold, J. J.; Cros, G. H.; Vian, L.; Tep, A.; Ramanadham, S.; Siou, G.; Diaz, J.; McNeill, J. H.; Serrano, J. J. *Pharmacol. Toxicol.* **1990**, *67*, 192.
(66) Thompson, K. H.; Leichter, J.; McNeill, J. H. *Biochem. Biophys. Res. Commun.* **1993**, *197*, 1549.
(67) Yuen, V. G.; Orvig, C.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **1993**, *71*, 263.
(68) Yuen, V. G.; Orvig, C.; Thompson, K. H.; McNeill, J. H. *Can. J. Physiol. Pharmacol.* **1993**, *71*, 270.
(69) Sakurai, H.; Taira, Z.; Sakai, N. *Inorg. Chim. Acta* **1988**, *151*, 85.
(70) Sakurai, H.; Tsuchiya, K.; Nukatsuka, M.; Kawada, J.; Ishikawa, S.; Yoshida, H.; Komatsu, M. *J. Clin. Biochem. Nutr.* **1990**, *8*, 193.
(71) Cam, M. C.; Faun, J.; McNeill, J. H. *Metabolism* **1994**, *44*, 332.
(72) Reul, B. A.; Amin, S. S.; Buchet, J.-P.; Ongemba, L. N.; Crans, D. C.; Brichard, S. M. *Brit. J. Pharm.* **1999**, *126*, 467.
(73) Marliss, E. B. *Metabolism* **1983**, *32 (Suppl. 1)*, 1.
(74) Penicaud, L.; Ferre, P.; Terretaz, J.; Kinebanyan, M. F.; Leturque, A.; Dore, E.; Girard, J.; Jeanrenaud, B.; Picon, L. *Diabetes* **1987**, *36*, 626.
(75) Mordes, J. P.; Rossini, A. A. *Am J. Med.* **1981**, *70*, 353.
(76) Shaver, A.; Ng, J. B.; Hall, D. A.; Soo Lum, B.; Posner, B. I. *Inorg. Chem.* **1993**, *32*, 3109.
(77) Stearns, D. M.; Armstrong, W. H. *Inorg. Chem.* **1992**, *31*, 5178.
(78) Evans, G. W.; Bowman, T. D. *J. Inorg. Biochem.* **1992**, *46*, 243.
(79) Shaver, A.; Ng, J. B.; Hynes, R. C.; Posner, B. I. *Acta Crystallogr.* **1994**, *C50*, 1044.
(80) Yale, J.-F.; Lachance, D.; Bevan, A. P.; Vigeant, C.; Shaver, A.; Posner, B. I. *Diabetes* **1995**, *44*, 1274.
(81) Yu, Z.-W.; Posner, B. I.; Smith, U.; Eriksson, J. W. *Biochim. Biophys. Acta* **1996**, *1310*, 103.
(82) Yu, Z.-W.; Jansson, P.-A.; Posner, B. I.; Smith, U.; Eriksson, J. W. *Diabetologia* **1997**, *40*, 1197.
(83) Djordjevic, C.; Vuletic, N.; Reslo, M. L.; Puryear, B. C.; Alimard, R. *Mol. Cell. Biochem.* **1995**, *153*, 25.
(84) Krejsa, C. M.; Nadler, S. G.; Esselstyn, J. M.; Kavanagh, T. J.; Ledbetter, J. A.; Schieven, G. L. *J. Biol. Chem.* **1997**, *272*, 11541.
(85) Crans, D. C.; Keramidas, A. D.; Hoover-Litty, H.; Anderson, O. P.; Miller, M. M.; Lemoine, L. M.; Pleasic-Williams, S.; Vandenberg, M.; Rossomoando, A. J.; Sweet, L. J. *J. Am. Chem. Soc.* **1997**, *119*, 5447.
(86) Messerschmidt, A.; Wever, R. *Proc. Natl. Acad. Sci. U.S.A.* **1996**, *93*, 392.
(87) Cornman, C. R.; Geiser-Bush, K. M.; Rowley, S. R.; Boyle, P. D. *Inorg. Chem.* **1997**, *36*, 6401.
(88) Bevan, A. P.; Burgess, J. W.; Yale, J.-F.; Drake, P. G.; LaChance, D.; Baquiran, G.; Shaver, A.; Posner, B. I. *Am. J. Physiol.* **1995**, *268*, E60.
(89) Melchior, M.; McNeill, J. H.; Orvig, C. Submitted for publication.
(90) Smith, C. J.; Rosen, O. M. In *Diabetes Mellitus: Theory and Practice*; Ellenberg, M.; Rifkin, M.; Eds.; McGraw-Hill: Toronto, 1983; p 89.
(91) Goldfine, A. B.; Simonson, D. C.; Folli, F.; Patti, M.-E.; Kahn, C. R. *J. Clin. Endocrinol. Metab.* **1995**, *80*, 3311.
(92) Cohen, N.; Halberstam, M.; Shlimovich, P.; Chang, C. J.; Shamoon, H.; Rossetti, L. *J. Clin. Invest.* **1995**, *95*, 2501.
(93) Rossetti, L.; Cohen, L.; Halberstam, M.; Shlimovich, P.; Hu, M.; Shamoon, H. *Can. J. Physiol. Phamacol.* **1994**, *72*, 11.
(94) Halberstam, M.; Cohen, N.; Shlimovich, P.; Chang, C. J.; Shamoon, H.; Rossetti, L. *J. Clin. Invest.* **1996**, *45*, 659.
(95) Goldfine, A. B.; Willsky, G. R.; Kahn, C. R. In *Vanadium Compounds: Chemistry, Biochemistry and Therapeutic Applications*; Tracey, A. S.. Crans, D. C., Eds.; ACS Symposium Series 711; Oxford University Press: New York, 1998; p 353.
(96) Thompson, K. H.; Battell, M.; McNeill, J. H. In *Vanadium in the Environment. Part 2. Health Effects*; Nriagu, J. O., Ed.; John Wiley & Sons: Ann Arbor, 1998, Vol. I, p 21.
(97) Ip, C. *J. Nutr.* **1998**, *128*, 1845.
(98) Mertz, W.; Abernathy, C. O.; Olin, S. S. *Risk Assessment of Essential Elements*; ILSI Press: Washington, DC, 1994.
(99) Curran, G. L.; Azarnoff, D. L.; Bolinger, R. E. *J. Clin. Invest.* **1959**, *38*, 1251.
(100) Dimond, E. G.; Caravaca, J.; Benchimol, A. *Am. J. Clin. Nutr.* **1963**, *12*, 49.
(101) Beattie, J. H.; Avenell, A. *Nutr. Res. Rev.* **1992**, *5*, 167.
(102) Stearns, D. M.; Belbruno, J. J.; Wetterhahn, K. E. *FASEB J.* **1995**, *9*, 1650.
(103) Pope, M. T.; Dale, B. W. *Chem. Soc. Q. Rev.* **1968**, *22*, 527.
(104) Coughlan, M. P.; *Molybdenum and Molybdenum-containing Enzymes*; Pergamon: Oxford, 1980.
(105) Palmer, G.; Olson, J. S. In *Molybdenum and Molybdenum-containing Enzymes*; Coughlan, M. P.; Ed.; Pergamon Press: Oxford, 1980; p 187.
(106) Stiefel, E. I. *Science* **1996**, *272*, 1599.
(107) Fillat, C.; Rodriguez-Gil, J. E.; Guinovart, J. J. *Biochem. J.* **1992**, *282*, 659.
(108) Goto, Y.; Kida, K.; Ikeuchi, M.; Kaino, Y.; Matsuda, H. *Biochem. Pharmacol.* **1992**, *44*, 174.
(109) Kadota, S.; Fantus, G.; Deragon, G.; Guyda, H. J.; Hersh, B.; Posner, B. I. *Biochem. Biophys. Res. Commun.* **1987**, *147*, 259.
(110) Heffetz, D.; Bushkin, I.; Dror, R.; Zick, Y. *J. Biol. Chem.* **1990**, *265*, 2896.
(111) Li, J.; Elberg, G.; Gefel, D.; Shechter, Y. *Biochemistry* **1995**, *34*, 6218.
(112) Ghiron, A. F.; Thompson, R. C. *Inorg. Chem.* **1990**, *29*, 4457.
(113) Guinovart, J. J.; Barbera, A.; Rodriguez-gil, J. E. U.S. Patent No. 5,595,763. 1997; *Chem. Abstr.* **1997**, *126*, 148554.
(114) Palanivel, R.; Sundravel, S.; Ravichandran, P.; Govindasamy, S. *Med. Sci. Res.* **1998**, *26*, 541.
(115) Thompson, K. H.; Scott, K. C.; Turnlund, J. R. *J. Appl. Physiol.* **1996**, *81*, 1404.
(116) Shisheva, A.; Gefel, D.; Shechter, Y. *Diabetes* **1992**, *41*, 982.
(117) Blostein-Fujii, A.; DiSilvestro, R. A.; Frid, D.; Katz, C.; Malarkey, W. *Am. J. Clin. Nutr.* **1997**, *66*, 639.
(118) Berg, J. M.; Shi, Y. *Science* **1996**, *271*, 1081.
(119) Thompson, K. H.; Godin, D. V. *Nutr. Res.* **1995**, *15*, 1377.
(120) Sandstead, H. H.; Egger, N. G. *Am J. Clin. Nutr.* **1997**, *66*, 681.
(121) Srivastava, P.; Saxena, A. K.; Kale, R. K.; Baquer, N. Z. *Res. Commun. Chem. Pathol. Pharmacol.* **1993**, *80*, 283.
(122) Paolisso, G.; Sgambato, S.; Pizza, G.; Passariello, N.; Varricchio, M.; D'Onofrio, F. *Diabetes Care* **1989**, *12*, 265.
(123) Thompson, K. H.; Mehr-Rahimi, B.; McNeill, J. H. *Magnes. Bull.* **1994**, *16*, 130.
(124) Mertz, W. *J. Nutr.* **1993**, *123*, 626.
(125) Rossetti, L.; Giaccari, A.; Klein-Robbenhaar, E.; Vogel, L. R. *Diabetes* **1990**, *39*, 1243.

CR980427C