Vanadium Compounds
Biological Actions and Potential as Pharmacological Agents
E. Tsiani and I.G. Fantus

Vanadium is an element found in low concentrations in mammals, for which a function remains to be discovered. Over the past century, vanadium compounds have been suggested anecdotally as therapeutic agents for a variety of diseases. The discovery that vanadate inhibits various enzymes, in particular protein tyrosine phosphatases, and mimics many of the biological actions of insulin suggested a potential role in the therapy of diabetes mellitus. Successful use and an enhancement of insulin sensitivity in rodents and human diabetic subjects, as well as the finding that these agents are capable of stimulating metabolic effects while bypassing the insulin receptor and the early steps in insulin action, target these agents preferentially toward type II diabetes mellitus. Long-term safety remains a major concern, as tissue accumulation and relative nonspecificity of enzyme inhibition may result in adverse effects. Continued research into mechanism of action, consequences of chronic administration, and improvement of specificity is warranted. Regardless of their ultimate success or failure as therapeutic agents, vanadium compounds continue to be useful probes of enzyme structure and function in various biological processes. (Trends Endocrinol Metab 1997;8:51–58). © 1997, Elsevier Science Inc.

Vanadium is a common element, which was discovered in 1830 by the chemist Nils Sefstrom. It was named after Vanadis, the Norse goddess of beauty, apparently because of the variety of bright and different colors that appear in its solutions upon changes in pH and concentration. Vanadium is an ultratrace element in mammals, and although several studies suggest that it is essential, at least in chicks and rats, its precise role in mammalian biology remains unknown. The interest of biologists and biochemists increased when vanadate was discovered to be an inhibitor of Na⁺, K⁺ ATPase found in commercial preparations of ATP from equine and rabbit skeletal muscle [reviewed by Nechay et al. (1986), Shechter (1990), Nielsen (1995)].

A series of landmark studies in 1979 and 1980 demonstrated the ability of Na orthovanadate (Na₃VO₄), Na metavanadate (NaV⁴O₄), and vanadyl sulfate (VOSO₄) to have insulin-like biological effects of glucose uptake and metabolism in skeletal muscle and adipose tissue in vitro (Tolman et al. 1979, Shechter and Karlish 1980, Dubyak and Kleinzeller 1980). These findings led to further interest in vanadium compounds, which was dramatically accelerated when Heyliger et al. (1985) first reported the successful treatment of streptozotocin-injected, insulin-deficient diabetic rats with oral vanadate. Blood glucose concentrations were lowered without a change in insulin concentrations, substantiating the ability of vanadate to mimic insulin in vivo. Since then, extensive studies exploring vanadium chemistry, including the synthesis of novel compounds, as well as the biological effects of vanadium on cells and tissues in vitro and in vivo, have been performed. Most recently, studies carried out in human subjects have demonstrated the insulin-mimetic potential of these agents. Some of these data are reviewed here, with a focus on the potential use of vanadium compounds as pharmacological agents. Owing to space limitations and the volume of published work, we regret that it is not possible to cite all original contributions to the literature.

- Chemistry

One of the most interesting and relevant features of the vanadate species is its similar structure (tetrahedral or trigonal bipyramidal) and charge to phosphate (Figure 1) (Shaver et al. 1995). It has been demonstrated that vanadate may form esteric linkages in an analogous manner to phosphate (Gresser and Tracey 1990), and this may be the basis for many of its biological effects, particularly enzyme inhibition. The existence of two major oxidation states, vanadate (V⁵⁺) and the reduced vanadyl IV (V⁴⁺), may complicate interpretation of some biological actions. A number of studies use vanadyl sulfate (IV), which is unstable in solution in the absence of GSH (glutathione) or 2-mercaptoethanol, being oxidized to vanadate. Intracellularly, however, most of the vanadate (V) is reduced to vanadyl (IV), which is bound to GSH and protein and is less potent as a phosphatase inhibitor (Macara et al. 1980, Elberg et al. 1994). Binding to NAD, GDP, ADP, and hydroxyl or thiol groups has been documented. These interactions can lead to a variety of biological effects, which will depend on the relative concentrations of the intracellular vanadium species and the various cellular constituents (for a detailed review of the chemistry, see Crans (1994), Crans et al. (1995)).

In an attempt to improve potential therapeutic efficacy, various vanadium compounds have been synthesized. One of the first and best studied is bis(maltolato) oxovanadium (IV). This "organо-

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E. Tsiani and I.G. Fantus are at the Department of Medicine, Mount Sinai Hospital, Department of Physiology and Banting and Best Diabetes Centre, University of Toronto, Ontario, M5G 1X5, Canada.
vanadium” compound, generated by forming bonds between two maltose sugars and the inner coordination sphere of the metal, promotes some stability, increases absorption, and apparently, at least in rodents, decreases GI toxicity (McNeill et al. 1992). More recently, a large number of peroxovanadium (pV) compounds have been synthesized (Posner et al. 1994). Peroxovanadium compounds are a powerful class of insulin-mimetic agents that were discovered when the two insulin-like agents, vanadate and H2O2 (hydrogen peroxide), were combined and found to be synergistic in their activities to produce insulin-like effects and activate the insulin receptor tyrosine kinase (IRK) (Kadota et al. 1987, Fantus et al. 1989). These compounds also resemble the phosphate structure (Figure 1), but the addition of peroxo group(s) sequentially increases their potency as protein tyrosine phosphatase inhibitors (see later here), presumably by increasing their abilities to oxidize irreversibly the bound thiols (Shaver et al. 1995).

**Figure 1.** Similarity of structure and charge among phosphate, vanadate, and peroxovanadate. From Shaver et al. (1995).

Enzyme inhibition

- P-type phosphorylated membrane ATPases, for example, Na+, K+ ATPase, Ca2+, Mg2+, ATPase
- Acid and alkaline phosphatases
- Phosphoprotein tyrosine phosphatases
- RNase
- Dynine ATPase
- Phosphoglucomutase, phosphoglycerate mutase
- Fructose-2, 6-bisphosphatase
- Glucose-6-phosphatase

**Table 1. The effect of vanadium compounds on enzyme activities**

<table>
<thead>
<tr>
<th>Enzyme inhibition</th>
<th>P-type phosphorylated membrane ATPases, for example, Na+, K+ ATPase, Ca2+, Mg2+, ATPase</th>
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<tbody>
<tr>
<td></td>
<td>Acid and alkaline phosphatases</td>
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<tr>
<td></td>
<td>Phosphoprotein tyrosine phosphatases</td>
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<td>RNase</td>
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<tr>
<td></td>
<td>Dynine ATPase</td>
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<tr>
<td></td>
<td>Phosphoglucomutase, phosphoglycerate mutase</td>
</tr>
<tr>
<td></td>
<td>Fructose-2, 6-bisphosphatase</td>
</tr>
<tr>
<td></td>
<td>Glucose-6-phosphatase</td>
</tr>
</tbody>
</table>

**Enzyme activation**

- Direct
  - Dehydrogenases: glucose-6-phosphate dehydrogenase
  - Epimerases: ribulose-5 phosphate epimerase
  - Isomerases: phosphoglucone isomerase
- Indirect
  - Adenylyl cyclase
  - Pyruvate kinase
  - Phosphoinositol kinase
  - Phospholipase Cγ

The alteration of enzyme activities, particularly inhibition, has been demonstrated in vitro to occur directly. Direct enzyme activation may occur by formation of phosphatelike esters with substrates. The indirect or secondary inhibition/activation of other enzymes, for example, tyrosine kinases via protein tyrosine phosphatase (PTP) inhibition, are numerous and not listed here.

**Metabolic Actions**

Virtually all of insulin’s bioeffects on glucose uptake and metabolism have been stimulated by vanadate in cultured cells and isolated tissues [reviewed by Shechter (1990), Posner et al. (1990)]. Although one report documents a glucagonlike (antiinsulin) effect to stimulate glycogen breakdown (Bosch et al. 1987), this was found at very high concentrations. Lower concentrations inhibited glucose output in perfused liver (Bruck et al. 1991) and in vivo (see later here). The effects of vanadate on fat and protein metabolism have not been eval-


**Nonmetabolic Actions**

The role of tyrosine phosphorylation in mitogenic signaling is well documented, and one would predict that vanadate may act as a growth stimulator. Indeed, many studies of cultured cells show a stimulation of cell growth in the presence of vanadate alone or an enhancement of mitogenesis when it is combined with growth factors [Klarlund (1985), Feldman et al. (1990), reviewed by Wang and Scott (1995)]. These studies often show enhanced tyrosine phosphorylation of various endogenous cellular proteins [Klarlund 1985, Brown and Gordon 1984] and/or activation of signaling molecules such as phosphatidylinositol-3-kinase (PI3K) [Chen et al. 1990, Tsiani et al. unpublished] and mitogen-activated protein kinase (MAPK) [D’Onofrio et al. 1994].

At the same time, however, a number of reports indicate that vanadate may inhibit cell proliferation, and vanadium compounds have been proposed as potential chemotherapeutic agents to inhibit tumor cell growth [Hanauske et al. (1987), Cruz et al. (1995), reviewed by Djordjevic (1995)]. The recent description of a number of tyrosine phosphatases, for example, SHP2/SYP (Milarski and Saltiel 1994, Xiao et al. 1994), and dual specific phosphatases, for example, cdc25 [Baratte et al. 1992], that are involved in the propagation of cell proliferation, combined with a relative lack of specificity of vanadate as a PTP inhibitor, provides a potential mechanism. Both vanadate [Hamaguchi et al. 1995] and pervanadate [Faure et al. 1995] interrupted the cell cycle at the G2/M phase in which the activation of the cyclin-dependent kinase (CDK1/cdc2)-cyclin B complex is dependent on cdc2 dephosphorylation by cdc25. Thus, the net effect of vanadate on cell growth in vivo will depend on the stage of the cell cycle, the relative concentrations of vanadate, and specific PTPs and, in tumor cells, particular mutations that may render a cell more or less responsive.

Other actions of vanadium compounds may also occur subsequent to PTP inhibition. Examples include activation of NADH oxidase in neutrophils [Grinstein et al. 1990], activation of the Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway [Haqu et al. 1995], a tyrosine kinase-dependent signaling system utilized by various hormones and cytokines [Roupas and Herington 1994], and alterations of receptor protein trafficking [Fantus et al. 1996].

**Actions in vivo**

Since the original description by Heylinger, a large number of studies in various rodent models of diabetes and/or insulin resistance have been performed to examine the ability of vanadium compounds to lower blood glucose and/or improve various characteristics of insulin-resistant states such as hyperlipidemia and hypertension (Table 2). In these models, elevated glucose concentrations were decreased, hypertriglyceridemia was improved, and in the spontaneously hypertensive rat (SHR) the development of hypertension was mitigated [see reviews by Shechter (1990), Orvig et al. (1995), Brichard and Henquin (1995)]. Although there has been some variability in success rates in the hands of different investigators, the effectiveness of these compounds, at least in rodents, is established. Many studies also demonstrate improvement or normalization of physiological and biochemical components of insulin action. Hepatic glucose output is decreased, and peripheral glucose uptake is enhanced. Enzyme activities, such as those of (G6Pase), fructose-2,6 bisphosphatase, and pyruvate kinase, as well as gene expression, have been normalized.

In some cases of streptozotocin-induced diabetes, withdrawal of vanadate did not result in a return of the diabetic state, suggesting that insulin secretory capacity may be preserved [Pederson et al. 1989]. These data, combined with the observation that vanadate administration lowered insulin requirements but could not completely replace insulin in the BB (biobreeding) Wistar diabetic rat [Orvig et al. 1995], suggest that in vivo some insulin is required for vanadate effectiveness. Thus, the clinical utility may be more relevant in insulin-resistant diseases such as type II diabetes than in type I diabetes.

Recently, two short-term studies in human subjects with diabetes mellitus have been completed [Goldfine et al. 1995, Cohen et al. 1995]. In five patients with insulin-dependent diabetes mellitus (IDDM, type I diabetes), there was an average 14% reduction in insulin requirements after 2 weeks of therapy with Na metavanadate (125 mg/day). Although changes in peripheral glucose uptake and hepatic glucose output were not observed in IDDM, in non–insulin-dependent diabetes mellitus (NIDDM, type II diabetes) there was an enhancement of peripheral insulin sensitivity.

**Table 2. Rodent models of diabetes and/or insulin resistance treated with vanadium compounds**

<table>
<thead>
<tr>
<th>Insulin deficient</th>
<th>Streptozotocin-injected rats</th>
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<tbody>
<tr>
<td>Pancreatectomized rats</td>
<td></td>
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<tr>
<td>BB Wistar rats</td>
<td></td>
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</table>

**Insulin resistant**

- ob/ob mice
- db/db mice
- fa/fa Zucker rats
- High sucrose fed rats
- Spontaneously hypertensive rats (SHR)
- Fructose-induced hypertensive rats

**BB, biobreeding.**
manifested as an increase in nonoxidative glucose disposal into muscle. In the second study, 3 weeks of treatment of six NIDDM subjects with vanadyl sulfate (100 mg/day) resulted in improvement in metabolic control, no change in fasting or stimulated insulin, and an increase in insulin-stimulated peripheral glucose uptake, particularly, enhanced glycogen synthesis, as well as inhibition of hepatic glucose production. These human studies are consistent with those in rodents (Myerovitch et al. 1991, Brichard et al. 1992, Eriksson et al. 1992) and in vitro (Fantus et al. 1990, Carey et al. 1995), which demonstrate not only direct insulin-mimetic actions but also enhancement of insulin sensitivity. In contrast to NIDDM, 3 weeks of vanadyl sulfate administration to obese nondiabetic subjects did not alter insulin sensitivity (Halberstam et al. 1996).

- **Mechanisms of Action**

  **Protein Tyrosine Phosphatase Inhibition**

  Most data support the inhibition of PTPs and resultant indirect stimulation of tyrosine phosphorylation as the mechanism by which vanadium compounds promote their insulinlike effects (Figure 2). Although one might suspect that the IR is involved, IR tyrosine phosphorylation is not consistently observed, and inhibition of the IR kinase does not abolish a number of effects on glucose metabolism (Shisheva and Shechter 1992) [for reviews of insulin action, see Cheatham and Kahn (1995), Quon et al. (1994)]. Although this is the case for vanadate, there is some evidence that peroxynitrate does not alter IR tyrosine phosphatase activity as PTP inhibitors, as well as more rapid entry into tissues. Vanadate may promote some insulinlike effects via activation of a 54-kD cytosolic tyrosine kinase in rat adipocytes (Shisheva and Shechter 1993). A number of such srclike tyrosine kinases exist, and it is plausible that similar downstream events are triggered by both the IR and such a tyrosine kinase or that such a kinase is a normal component of the insulin action pathway. Some bioeffects, such as lipogenesis, appeared to be mediated via this pathway whereas others, such as glucose transport, were not. Furthermore, vanadate, although a nonspecific PTP inhibitor, was found to be 10-fold more efficacious in inhibiting cytosolic PTP activity (20 μM) as compared with that in the particulate fraction (200 μM) (Goldfine et al. 1995). Another group of tyrosine kinases, the JAKs, have recently been found to phosphorylate the major IR substrates, IRS-1 and IRS-2 [reviewed by Waters and Pessin (1996)] and may also function to transmi vanadate actions. Thus, there are likely differences in the signaling pathways utilized for the various insulinlike bioeffects and possibly different but overlapping mechanisms for the various vanadium compounds.

  The ability of vanadate to inhibit PTPs allows the use of this agent to probe the role of PTP activity in the physiology of insulin action and resistance. The net hormonal signal will depend on the balance between receptor tyrosine kinase (RTK) and PTP activities. In the case of insulin, there are "spare" receptors so that maximum response is achieved at subsaturating concentrations of hormone [reviewed by Kahn (1978)]. The augmentation of RTK activity by vanadate in such a circumstance would lead to an apparent increase in sensitivity to insulin, as well as to a prolongation of insulin action (Fantus et al. 1994). Thus, vanadate would render lower concentrations of endogenous or exogenous insulin more effective.

  The hypothesis that abnormally elevated PTP activity is responsible for some forms of insulin resistance is supported by the finding that increased IR tyrosine dephosphorylating activity was found in adipocytes of obese subjects (Ahmad et al. 1995). Exposure of cultured cells to vanadate was more effective in correcting insulin-stimulated IR...
Downstream Effects

Apart from the indirect activation of tyrosine kinases, vanadate may stimulate signals downstream of the IR and IRS-1, such as MAPK and PI3K. Activation of MAPK may also occur indirectly by inhibition of the vanadate-sensitive dual-specific MAPK phosphatase (MKP-1), whereas PI3K activation may be mediated subsequent to tyrosine kinase activation. Although PI3K activation is required for insulin-stimulated glucose transport (Cheatham and Kahn 1995), the authors (unpublished) and others (Ida et al. 1996) found that vanadate and pV can stimulate glucose transport in the presence of PI3K inhibition by wortmannin. The stimulation of glucose transport by both insulin and vanadium compounds was blocked by cytochalasin D, which disrupts the actin cytoskeleton, indicating that the two pathways converge (Tsiani and Fantus unpublished). Furthermore, the combination of a tyrosine kinase inhibitor with wortmannin blocked pV stimulation of glucose transport (Ida et al. 1996). These data indicate that a tyrosine kinase signaling pathway that is independent of PI3K and not utilized by insulin can lead to glucose transport stimulation. This “bypass” pathway may contribute to vanadate’s effectiveness in insulin-resistant diseases, and the elucidation of its biochemical components could lead to the design of novel therapeutic agents for NIDDM (Figure 3).

Other Contributions to Glucose-Lowering Action

Two other effects of vanadate may contribute to its glucose-lowering action. It has been noted that oral administration of vanadate may decrease food and fluid intake. Although most studies could not attribute vanadate’s effectiveness merely to a decrease in food intake, and in the short-term studies in humans, no significant changes in food intake were found; this issue remains controversial [see Domingo et al. (1994), McNeill et al. (1994), Williams and Malabu (1994)]. Furthermore, intracerebroventricular administration of vanadate decreased food intake in rats (Meyerovitch et al. 1989), and insulin crossing the blood-brain barrier has been demonstrated to contribute to satiety (Schwartz et al. 1992). It is intriguing to wonder whether any form of oral vanadate may access the central nervous system (CNS) to exert insulin-like effects. Another action of vanadate is to inhibit intestinal glucose absorption (Madsen et al. 1993); however, it is unlikely that at therapeutic doses this effect plays a major role.

Side Effects and Toxicity

In early studies in rodents, decreased fluid and food intake and diarrhea were the major toxic effects and led to increased morbidity and mortality [reviewed by Domingo et al. (1995)]. Introduction of the agents at low doses with a gradual increase to maintenance levels prevents these effects. In humans, both vanadyl sulfate and Na metavanadate resulted in some nausea, which responded to a decrease in dose. Studies in rodents suggest that modification of the vanadium species with organic ligands may decrease the GI side effects and enhance absorption, resulting in an apparent increased potency (Yuen et al. 1993). Peroxovanadium compounds are more potent PTP inhibitors but are degraded in the gastric acidic environment (Yale et al. 1995); however, modification and testing of many ligands is under way. Long-term administration of vanadate and vanadyl sulfate to rats has not been associated in most studies with any major toxicity (Nielsen 1995). At higher doses, there have been reduced sperm counts in male rats (Domingo et al. 1995). Other studies reported decreased hemoglobin levels in rats and Cohen et al. (1995) noted a very small drop in hematocrit in treated human subjects that was maintained for 2 weeks after discontinuation of treatment. One other important adverse effect has been embryotoxicity in rats and mice (Domingo et al. 1995, Leonard and Gerber 1994). This has great clinical implications, as one might expect fetal tissues to be more sensitive, and these agents would not likely be considered for use during pregnancy or lactation.

A most important concern in the long-term use of any drug is carcinogenic
potential. Although there is no evidence in long-term studies in rats or in humans exposed to vanadium of an increased incidence of neoplasms, the known role of tyrosine kinases in mitogenesis and the many in vitro studies documenting the ability of vanadate to stimulate growth or potentiate the effects of growth factors indicates that this issue must be carefully evaluated. It is not known whether under certain circumstances oral vanadium compounds may promote or enhance tumor growth.

An additional measure of potential long-term toxicity is accumulation in tissues. In treated rats, vanadium accumulated mostly in kidney, spleen, testes, liver, and bone (Hamel and Duckworth 1995, Domingo et al. 1995). Presumably, some equilibrium is reached in most soft tissues that do not store vanadium; however, its similarity to phosphate could result in a continuous accumulation in bone. Whether this is of clinical relevance remains to be determined.

**Conclusion and Future Directions**

Vanadium compounds have been extremely useful as probes of enzyme structure and function and of the role of tyrosine phosphorylation in cellular signaling. The ability of these agents in vivo to mimic insulin and enhance its metabolic actions with relatively few adverse effects has sparked interest in their potential as pharmacological agents. Several questions remain to be addressed. An increased understanding of the molecular mechanisms by which vanadate exerts its actions is required. This will not only better define the potential use of these agents for particular patients but also elucidate novel signaling pathways to metabolic actions. Physiological studies of vanadate effects on protein metabolism, an important component of insulin action, are lacking. Additional studies are also needed to document the putative inhibitory effects on cell growth and proliferation in vivo. Compared with insulin and insulin-like growth factor-1 (IGF-1), vanadate could present an augmented ratio of metabolic to mitogenic stimulation. Clearly, further work is required to increase our confidence that there are no serious long-term adverse effects. Synthesis of new compounds modified by different ligands may enhance specificity and potency. At this point, the utility of these agents appears to be most promising for insulin-resistant subjects with forms of type II diabetes.

**Acknowledgment**

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**References**


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