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 (Na_3VO_4), Na metavanadate ($NaVO_3$), and vanadyl sulfate ($VOSO_4$) to have insulinlike 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 insulinmimetic 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 V (+5) and the reduced vanadyl IV (+4), 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 "organo-

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, , Toronto, Ontario, M5G 1X5, Canada.

 © 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7

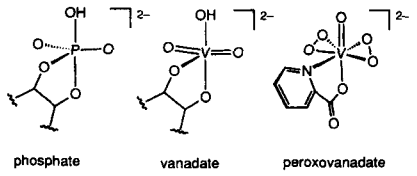

Figure 1. Similarity of structure and charge among phosphate, vanadate, and peroxovanadate. From Shaver et al. (1995).

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 insulinlike agents, vanadate and H_2O_2 (hydrogen peroxide), were combined and found to be synergistic in their activities to produce insulinlike 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).

• Enzyme Interactions

Based on knowledge of its chemistry, one might expect vanadate to alter the activities of many enzymes, in particular those involved in splitting a phosphate ester linkage (Table 1). Early work documented the effect of vanadate to inhibit the "P"-type phosphorylated ATPases such as Na^+, K^+ ATPase, and Ca^{2+}, Mg^{2+} ATPase. This has been demonstrated directly in vitro and involves the formation of a relatively stable vanadoenzyme transition state analogue. Subsequently, vanadate has been found to inhibit a number of phosphatases, including acid and alkaline phosphatases, as well as protein tyrosine phosphatases (PTPs). The latter observation, made during the time when the importance of tyrosine phosphorylation in cellular function was recognized (Hunter 1987), led to great interest in using vanadate both as a pharmacological probe as well as a standard ingredient of cell and tissue isolation buffers to preserve endogenous levels of protein tyrosine phosphorylation. Furthermore, the cloning and sequencing of the insulin receptor (IR) and its recognition as a tyrosine protein kinase (Ullrich et al. 1985) strongly supported the concept that PTP inhibition was relevant to most, if not all, of the insulin-mimetic actions of vanadate. It should be noted that vanadate does not inhibit serine/threonine phosphatases. Other enzymes that are inhibited by vanadate include RNase, dynein ATPase, phosphoglucomutase, and glucose-6-phosphatase (G6Pase) [reviewed by Stankiewicz et al. (1995)]. It has been suggested that inhibition of G6Pase may contribute to the glucose-lowering action of vanadate in vivo (Schulz et al. 1988).

In contrast to the inhibition of most enzymes with which it interacts, vanadate has been reported to activate adenylyl cyclase. This was first demonstrated in rat adipocyte membranes (Schwabe et al. 1979). This effect may be indirect, mediated by an interaction of GDP-V with G (GTP binding) proteins to activate the enzyme or mediated by oligomers as high (mM) concentrations are required (Stankiewicz and Tracey 1995). Although this action in adipocytes would be expected to stimulate lipolysis, vanadate treatment of intact adipocytes results in an antilipolytic action (Shechter 1990, Fantus et al. 1989). A vanadyl sulfate-GSH solution inhibits purified cAMP-dependent protein kinase (Brownsey and Dong 1995). Whether or not this latter action is involved in the insulinlike antilipolytic effect of vanadate is not clear; however, this illustrates that caution is required when extrapolating from in vitro to in vivo actions.

Table 1. The effect of vanadium compounds on enzyme activities

Enzyme inhibition
P-type phosphorylated membrane ATPases, for example, Na^+, K^+ ATPase, Ca^{2+}, Mg^{2+}, ATPase
Acid and alkaline phosphatases
Phosphoprotein tyrosine phosphatases
RNase
Dynein ATPase
Phosphoglucomutase, phosphoglycerate mutase
Fructose-2, 6-bisphosphatase
Glucose-6-phosphatase
Enzyme activation
Direct
Dehydrogenases: glucose-6-phosphate dehydrogenase
Epimerases: ribulose-5 phosphate epimerase
Isomerases: phosphoglucose isomerase
Indirect
Adenylyl cyclase
Pyruvate kinase
Phosphoinositide 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-

© 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7

uated in as much detail. Vanadate effectively inhibits lipolysis and stimulates lipogenesis in adipocytes (Fantus et al. 1989, Shechter 1990) and mimics insulin in isolated hepatocytes to inhibit very low density lipoprotein (VLDL) release (Jackson et al. 1988). The anabolic effect of insulin on protein metabolism in skeletal muscle is not mimicked by vanadate in vitro (Clark et al. 1985). There appears to be stimulation of protein synthesis in adipocytes by the more potent peroxovanadium (Fantus et al. 1989), but in contrast to glucose metabolic effects, this was still not as effective as insulin. The reason(s) for this discrepancy is not clear, but one possibility is that this signal transduction pathway involves a PTP. Thus, the net action on a given signaling pathway will depend on the relative amounts and activities of the various PTPs involved in that pathway and their relative sensitivities to inhibition by vanadate. A similar phenomenon may explain the variable results observed in regard to vanadate's effect on amino acid transport. Although some have found stimulation of uptake in muscle (Munoz et al. 1992), vanadate inhibits amino acid uptake in intestinal cells (Hajjar et al. 1989) and in cultured rat L6 myotubes (Tsiani et al. 1996).

- **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, cdc 25 (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 Heyliger, 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.

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

Insulin deficient
Streptozotocin-injected rats
Pancreatectomized rats
BB Wistar rats
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.

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

© 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7

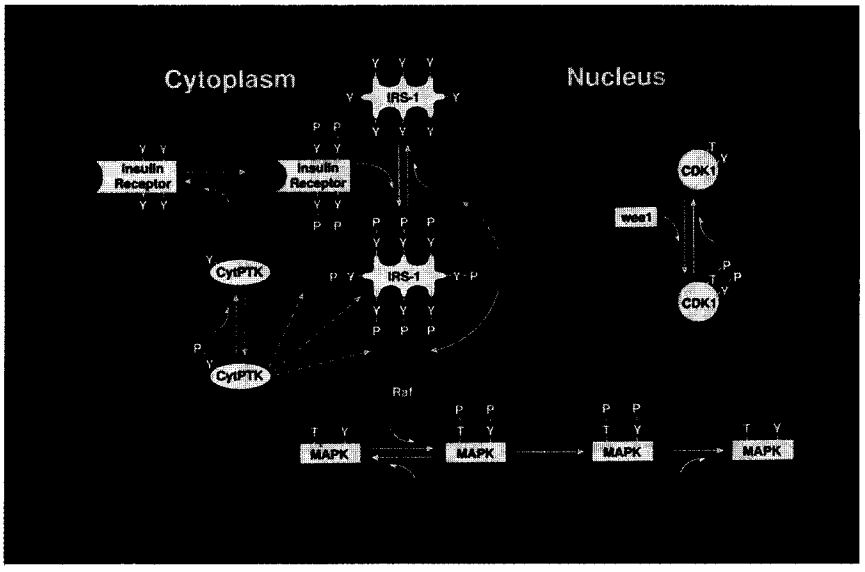

Figure 2. Sites of potential protein tyrosine phosphatase (PTP) inhibition by vanadium compounds. Insulin signal transduction proceeds via receptor autophosphorylation on tyrosine (tyr) followed by tyr phosphorylation of IRS-1 and/or IRS-2. The specific PTPs responsible for dephosphorylation of the insulin receptor (IR) and IRS-1 in vivo are not defined. Signaling proteins bind to tyrosine phosphorylated IRS-1 via their SH2 (src homology 2) domains, which results in activation, for example, phosphatidylinositol-3-kinase (PI3K), growth factor receptor bound protein 2 (GRB 2) and SH2 domain containing tyr phosphatase 2 (SHP 2). SHP2 may contribute to IRS-1 dephosphorylation but has been shown to participate in the mitogenic response to insulin. PI3K participates in the insulin-stimulated glucose transport response. GRB2, in a complex with the guanine nucleotide exchange factor mSOS (mammalian son of sevenless), stimulates ras activation and the mitogen-activated protein kinase (MAPK) pathway. This pathway is also (and perhaps preferentially) triggered by tyr phosphorylation of SHC (src homology and collagenlike protein), which also binds GRB2 (not shown). MAPK activation by vanadate may not require ras activation but may occur indirectly via inhibition of cytosolic (MKP-1) and/or nuclear (PAC-1) dual specificity phosphatases. Cytosolic tyr kinases are also activated by vanadate presumably by inhibition of cytosolic (cyt) PTPs. These cyt PTKs may signal similar and or additional SH2 domain containing protein pathways. In the nucleus, inhibition of other dual specificity phosphatases such as cdc25 may result in disruption of the cell cycle. CDK, cyclin-dependent kinase. The phosphatases inhibited by vanadate are depicted by the *red boxes*. Modified from Fantus et al. (1995).

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 pervanadate and pV compounds do act predominantly via the IR [Posner et al. (1994), unpublished data]. This may relate to differences in potency and specificity 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 src-like tyrosine kinases exist, and it is plausible that several 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 transmit 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

© 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7

tyrosine (tyr) kinase activity in tumor necrosis factor-α (TNF α)-induced insulin resistance than in that caused by hyperglycemia (Kroder et al. 1996). These studies raise the possibility that vanadium compounds could be targeted to certain patients within the type II diabetes category.

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).

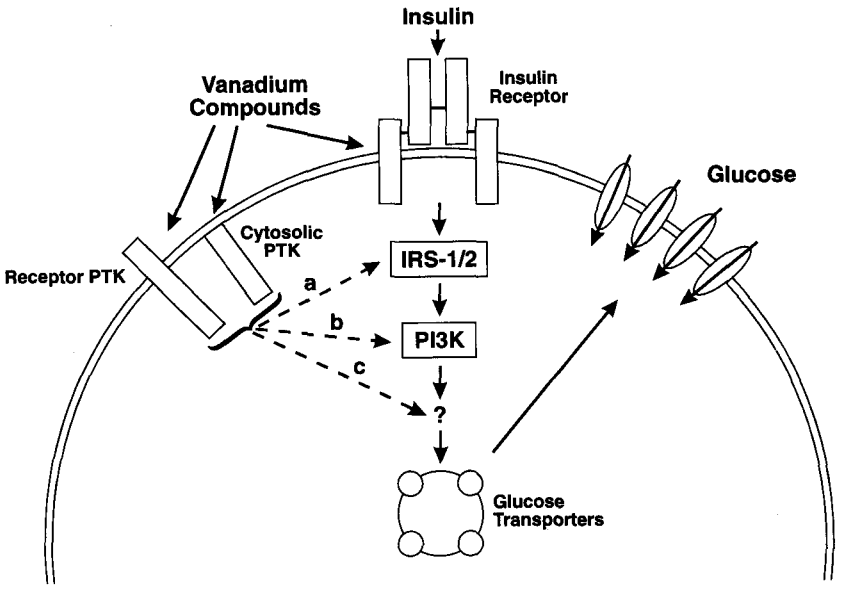

Figure 3. Stimulation of glucose transport by vanadium compounds. Insulin stimulation of glucose transport is mediated via tyrosine (tyr) phosphorylation of IRS 1 and/or IRS-2 and subsequent activation of PI3K. The events linking PI3K to insulin-sensitive glucose transporter vesicle translocation to the plasma membrane remain to be defined. Vanadate may stimulate glucose transport via alternate pathways that appear to require tyrosine phosphorylation but not necessarily that of the IR or IRS-1. In addition, glucose transport stimulation may occur, at least in part, by mechanisms that do not involve PI3K. The molecular components of the "bypass" pathways a, b, and c remain to be defined. Insulin resistance caused by defects at sites proximal to the point of convergence *(?)* may be overcome by vanadium compounds and novel drugs designed to stimulate these pathways.

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 insulinlike 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

 © 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7

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

The authors would like to thank Drs. B. Posner, A. Shaver, T. Cruz, and M. Tyers for helpful discussions and sharing of data. The research of the authors cited was supported by grants from the Medical Research Council of Canada (MT-7658) to IGF and a University of Toronto Open Studentship (ET). The authors thank Mrs. B. Baubinas for excellent secretarial assistance.

References

Ahmad F, Considine RV, Goldstein BJ: 1995. Increased abundance of the receptor-type protein-tyrosine phosphatase LAR accounts for the elevated insulin receptor dephosphorylating activity in adipose tissue of obese human subjects. J Clin Invest 95:2806–2812.

Baratte B, Meijer L, Galaktionov K, Beach D: 1992. Screening for antimitotic compounds using the cdc25 tyrosine phosphatase, an activator of the mitosis-inducing $p34^{cdc2}$/cyclin B^{cdc13} protein kinase. Anticancer Res 12:873–880.

Bosch F, Arino J, Gomez-Foix AM, Guinovart JJ: 1987. Glycogenolytic noninsulin-like effects of vanadate on rat hepatocyte glycogen synthase and phosphorylase. J Biol Chem 262:218–222.

Brichard SM, Henquin J-C: 1995. The role of vanadium in the management of diabetes. Trends Pharmacol Sci 16:265–270.

Brichard SM, Ongemba LN, Henquin JC: 1992. Oral vanadate decreases muscle insulin resistance in obese fa/fa rats. Diabetologia 35: 522–527.

Brown D, Gordon J: 1984. The stimulation of $pp60^{v\text{-}src}$ kinase activity by vanadate in intact cells accompanies a new phosphorylation state of the enzyme. J Biol Chem 259:9580–9686.

Brownsey RW, Dong GW: 1995. Evidence for selective effects of vanadium on adipose cell metabolism involving actions on cAMP-dependent protein kinase. Mol Cell Biochem 153:131–137.

Bruck R, Prigozin H, Krepel Z, Rotenberg P, Shecter Y, Bar-Meir S: 1991. Vanadate inhibits glucose output from isolated perfused rat liver. Hepatology 14:540–544.

Carey JD, Azevedo JL, Morris PG, Pories WJ, Dohm GL: 1995. Okadaic acid, vanadate, and phenylarsine oxide stimulate 2-deoxyglucose transport in insulin-resistant human skeletal muscle. Diabetes 44:682–688.

Cheatham B, Kahn CR: 1995. Insulin action and the insulin signaling network. Endocr Rev 16:117–142.

Chen YX, Yang DC, Brown AB, Jeng Y, Tatoyan A, Chan TM: 1990. Activation of a membrane-associated phosphatidylinositol kinase through tyrosine-protein phosphorylation by naphthoquinones and orthovanadate. Arch Biochem Biophys 283:184–192.

Clark AS, Fagan JM, Mitch WE: 1985. Selectivity of insulin-like actions of vanadate on glucose and protein metabolism in skeletal muscle. Biochem J 232:273–276.

Cohen N, Halberstam M, Shlimovich, Chang CJ, Shamoon H, Rossetti L: 1995. Oral vanadyl sulfate improves hepatic and peripheral insulin sensitivity in patients with non–insulin-dependent diabetes mellitus. J Clin Invest 95:2501–2509.

Crans DC: 1994. Aqueous chemistry of labile oxovanadates: relevance to biological studies. Comments Inorganic Chem 16:1–33.

Crans DC, Mahroof-Tahir M, Keramidas AD: 1995. Vanadium chemistry and biochemistry of relevance for use of vanadium compounds as anti-diabetic agents. Mol Cell Biochem 153:17–24.

Cruz TF, Morgan A, Min W: 1995. In vitro and in vivo antineoplastic effects of orthovanadate. Mol Cell Biochem 153:161–166.

Djordjevic C: 1995. Antitumor activity of vanadium compounds. *In* Sigel H, Sigel A, ed. Metal Ions in Biological Systems. New York, Marcel Dekker, 31:595–616.

Domingo JL, Gomez M, Sanchez OJ, Llobet JM, Keen CL: 1994. Relationship between reduction in food intake and amelioration of hyperglycemia by oral vanadate in STZ-induced diabetic rats. Diabetes 43:1267.

Domingo JL, Gomez M, Sanchez OJ, Llobet JM, Keen CL: 1995. Toxicology of vanadium compounds in diabetic rats: the action of chelating agents on vanadium accumulation. Mol Cell Biochem 153:233–240.

D'Onofrio F, Le MQ, Chiasson J-L, Srivastava AK: 1994. Activation of mitogen-activated protein (MAP) kinases by vanadate is independent of insulin receptor autophosphorylation. FEBS Lett 340:269–275.

Dubyak GR, Kleinzeller A: 1980. The insulin-mimetic effects of vanadate in isolated rat adipocytes. Dissociation from effects of vanadate as $(Na^+\text{-}K^+)$-ATPase inhibitor. J Biol Chem 255:5306–5312.

Elberg G, Li J, Shechter Y: 1994. Vanadium activates or inhibits receptor and non-receptor protein tyrosine kinases in cell-free experiments, depending on its oxidation state: possible role of endogenous vanadium in controlling cellular protein tyrosine kinase activity. J Biol Chem 269:9521–9527.

© 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7

Eriksson JW, Lonnroth P, Smith U: 1992. Vanadate increases cell surface insulin binding and improves insulin sensitivity in both normal and insulin-resistant rat adipocytes. Diabetologia 35:510–516.

Fantus IG, Deragon G, Lai R, Tang S: 1995. Modulation of insulin action by vanadate: evidence of a role for phosphotyrosine phosphatase activity to alter cellular signaling. Mol Cell Biochem 153:103–112

Fantus IG, Kadota S, Deragon G, Foster B, Posner BI: 1989. Pervanadate [peroxide(s) of vanadate] mimics insulin action in rat adipocytes via activation of the insulin receptor tyrosine kinase. Biochemistry 28:8864–8871.

Fantus IG, Ahmad F, Deragon G: 1990. Vanadate augments insulin binding and prolongs insulin action in rat adipocytes. Endocrinology 127:2716–2725.

Fantus IG, Ahmad F, Deragon G: 1994. Vanadate augments insulin-stimulated insulin receptor kinase activity and prolongs insulin action in rat adipocytes: evidence for transduction of amplitude of signaling into duration of response. Diabetes 43:375–383.

Fantus IG, George R, Tang S, Chong P, Poznansky MJ: 1996. The insulin-mimetic agent vanadate promotes receptor endocytosis and inhibits intracellular ligand-receptor degradation by a mechanism distinct from the lysosomotropic agents. Diabetes 45:1084–1093.

Faure R, Vincent M, Dufour M, Shaver A, Posner BI: 1995. Arrest at the G2/M transition of the cell cycle by protein-tyrosine phosphatase inhibition: studies on a neuronal and a glial cell line. J Cell Biochem 59:389–401.

Feldman RA, Lowy DR, Vass WC: 1990. Selective potentiation of C-fps/fes transforming activity by a phosphatase inhibitor. Oncogene Res 5:187–197.

Goldfine AB, Simonson DC, Folli F, Patti M-E, Kahn CR: 1995. In vivo and in vitro studies of vanadate in human and rodent diabetes mellitus. Mol Cell Biochem 153:217–231.

Gresser MJ, Tracey AS: 1990. Vanadates as phosphate analogs in biochemistry. *In* Chasteen ND, ed. Vanadium in Biological Systems. Dordrecht, Kluwer Academic, pp 63–79.

Grinstein S, Furuya W, Lu DJ, Mills BG: 1990. Vanadate stimulates oxygen consumption and tyrosine phosphorylation in electropermeabilized human neutrophils. J Biol Chem 265:318–327.

Hajjar JJ, Dobish MP, Tomicic TK: 1989. Effect of chronic vanadate ingestion on amino acid and water absorption in rat intestine. Arch Toxicol 63:29–33.

Halberstam M, Cohen N, Shlimovich P, Rossetti L, Shamoon H: 1996. Oral vanadyl sulfate improves insulin sensitivity in NIDDM but not in obese nondiabetic subjects. Diabetes 45:659–666.

Hamaguchi T, Sudo T, Osada H: 1995. RK-682, a potent inhibitor of tyrosine phosphatase arrested the mammalian cell cycle progression at G1 phase. FEBS Lett 372:54–58.

Hamel FG, Duckworth WC: 1995. The relationship between insulin and vanadium metabolism in insulin target tissues. Mol Cell Biochem 153:95–102.

Hanauske U, Hanauske A-R, Marshall MH, Muggia VA, Von Hoff DD: 1987. Biphasic effect of vanadium salts on in vitro tumor colony growth. Intern J Cell Cloning 5:170–178.

Haqu SJ, Flati V, Deb A, Williams BRG: 1995. Role of protein-tyrosine phosphatases in stat 1α-mediated cell signaling. J Biol Chem 270: 25,709–25,714.

Heyliger CE, Tahiliani AG, McNeill JH: 1985. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science 227:1474–1477.

Hunter T: 1987. A thousand and one protein kinases. Cell 50:823–829.

Ida M, Imai K, Hashimoto S, Kawashima H: 1996. Pervanadate stimulation of wortmannin-sensitive and -resistant 2-deoxyglucose transport in adipocytes. Biochem Pharmacol 51:1061–1067.

Jackson TK, Salhanick AI, Sparks JD, Sparks CE, Bolognino M, Amatruda JM: 1988. Insulin-mimetic effects of vanadate in primary cultures of rat hepatocytes. Diabetes 37: 1234–1240.

Kadota S, Fantus IG, Deragon G, Guyda HJ, Hersh B, Posner BI: 1987. Peroxide(s) of vanadium: a novel and potent insulin-mimetic agent which activates the insulin receptor kinase. Biochem Biophys Res Commun 147:259–266.

Kahn CR: 1978. Insulin resistance, insulin insensitivity, and insulin unresponsiveness: a necessary distinction. Metabolism 27:1893–1902.

Klarlund JK: 1985. Transformation of cells by an inhibitor of phosphatases acting on phosphotyrosine proteins. Cell 41:707–717.

Kroder G, Bossenmaier B, Kellerer M, et al.: 1996. Tumor necrosis factor-α- and hyperglycemia-induced insulin resistance: evidence for different mechanisms and different effects on insulin signaling. J Clin Invest 97: 1471–1477.

Leonard A, Gerber GB: 1994. Mutagenicity, carcinogenicity and teratogenicity of vanadium compounds. Mutat Res 317:81–88.

Macara IG, Kustin K, Cantley LC, Jr.: 1980. Glutathione reduces cytoplasmic vanadate: Mechanism and physiological implications. Biochim Biophys Acta 629:95–106.

Madsen KL, Porter VM, Fedorak LN: 1993. Oral vanadate reduces Na^+-dependent glucose transport in rat small intestine. Diabetes 42:1126–1132.

McNeill JH, Yuen VG, Hoveyda HR, Orvig C: 1992. Bis(maltolato) oxo vanadium (IV) is a potent insulin mimic. J Med Chem 35:1489–1491.

McNeill JH, Battell M, Cam M, Dai S, Thompson K, Yuen V: 1994. Oral vanadium and lowering of blood glucose. Diabetes 43:1268.

Meyerovitch J, Shechter Y, Amir S: 1989. Vanadate stimulates in vivo glucose uptake in brain and arrests food intake and body weight gain in rats. Physiol Behav 45:1113–1116.

Meyerovitch J, Rothenberg P, Shechter Y, Bonner-Weir S, Kahn CR: 1991. Vanadate normalizes hyperglycemia in two mouse models of non–insulin-dependent diabetes mellitus. J Clin Invest 87:1286–1294.

Milarski KL, Saltiel AR: 1994. Expression of catalytically inactive syp phosphatase in 3T3 cells blocks stimulation of mitogen-activated protein kinase by insulin. J Biol Chem 269: 21,239–21,243.

Munoz P, Guma A, Camps M, et al.: 1992. Vanadate stimulates system A amino acid transport activity in skeletal muscle. J Biol Chem 267:10,281–10,388.

Nechay BR, Nanninga LB, Nechay PSE, et al.: 1986. Role of vanadium in biology. Fed Proc 45:123–132.

Nielsen FH: 1995. Vanadium in mammalian physiology and nutrition. *In* Sigel H, Sigel A, eds. Metal Ions in Biological Systems. New York, Marcel Dekker, 31:543–574.

Orvig C, Thompson KH, Battell M, McNeill JH: 1995. Vanadium compounds as insulin mimics. *In* Sigel H, Sigel A, eds. Metal Ions in Biological Systems. New York, Marcel Dekker, 31:575–594.

Pederson RA, Ramanadham S, Buchan AMJ, McNeill JH: 1989. Long-term effects of vanadyl treatment on streptozotocin-induced diabetes in rats. Diabetes 38:1390–1395.

Posner BI, Shaver A, Fantus IG: 1990. Insulin mimetic agents: vanadium and peroxovanadium compounds. *In* Bailey CJ, Flatt PR, ed. New Antidiabetic Drugs. London, Smith-Gordon, pp 107–118.

Posner BI, Faure R, Burgess JW, et al.: 1994. Peroxovanadium compounds: a new class of potent phosphotyrosine phosphatase inhibitors which are insulin mimetics. J Biol Chem 269:4596–4604.

Quon MJ, Butte AJ, Taylor SI: 1994. Insulin signal transduction pathways. Trends Endocrinol Metab 5:369–376.

Roupas P, Herington AC: 1994. Postreceptor signaling mechanisms for growth hormone. Trends Endocrinol Metab 5:154–158.

Schulz LO: 1988. Suppression of the hepatic glucose-6-phosphatase system in diabetic rats by vanadate. Ann Nutr Metab 32:289–296.

Schwabe U, Puckstein C, Hammerman H, Sochtig E: 1979. Activation of adenylate cyclase by vanadate. Nature 277:143–145.

Schwartz MW, Figlewicz DP, Baskin DG, Woods SC, Porte D, Jr.: 1992. Insulin in the

 © 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7

brain: a hormonal regulator of energy balance. Endocr Rev 13:387–414.

Shaver A, Ng JB, Hall DA, Posner BI: 1995. The chemistry of peroxovanadium compounds relevant to insulin mimesis. Mol Cell Biochem 153:5–15.

Shechter Y: 1990. Insulin mimetic effects of vanadate: possible implications for future treatment of diabetes. Diabetes 39:1–5.

Shechter Y, Karlish SJD: 1980. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (IV) ions. Nature 284:556–558.

Shisheva A, Shechter Y: 1992. Quercetin selectively inhibits insulin receptor function in vitro and the bioresponses of insulin and insulinomimetic agents in rat adipocytes. Biochemistry 31:8059–8063.

Shisheva A, Shechter Y: 1993. Role of cytosolic tyrosine kinase in mediating insulin-like actions of vanadate in rat adipocytes. J Biol Chem 268:6463–6469.

Stankiewicz PJ, Tracey AS: 1995. Stimulation of enzyme activity by oxovanadium complexes. *In* Sigel H, Sigel A, eds. Metal Ions in Biological Systems. New York, Marcel Dekker, 31: 249–286b.

Stankiewicz PJ, Tracey AS, Crans DC: 1995. Inhibition of phosphate-metabolizing enzymes by oxovanadium complexes. *In* Sigel H, Sigel A, eds. Metal Ions in Biological Systems. New York, Marcel Dekker, 31:287–324a.

Tolman EL, Barris E, Burns M, Pansini A, Partridge R: 1979. Effects of vanadium on glucose metabolism in vitro. Life Sci 25:1159–1164.

Tsiani E, Abdullah N, Fantus IG: 1997. The insulin-mimetic agents vanadate and pervanadate stimulate glucose but inhibit amino acid uptake. Am J Physiol 272:C156–C162.

Ullrich A, Bell JR, Chen EY, et al.: 1985. Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 313:756–761.

Wang H, Scott RE: 1995. Unique and selective mitogenic effects of vanadate on SV40-transformed cells. Mol Cell Biochem 153:59–67.

Waters SB, Pessin JE: 1996. Insulin receptor substrate 1 and 2 (IRS1 and IRS2): what a tangled web we weave. Trends Cell Biol 6:1–4.

Williams G, Malabu U: 1994. Reply to Domingo et al and McNeil et al. Diabetes 43:1269–1270.

Xiao S, Rose DW, Sasaoka T, et al.: 1994. Syp (SH-PTP2) is a positive mediator of growth factor-stimulated mitogenic signal transduction. J Biol Chem 269:21,244–21,248.

Yale JF, Lachance D, Bevan AP, Vigeant C, Shaver A, Posner BI: 1995. Hypoglycemic effects of peroxovanadium compounds in Sprague-Dawley and diabetic BB rats. Diabetes 44:1274–1279.

Yuen VG, Orvig C, McNeill JH: 1993. Glucose lowering effects of a new vanadium complex, bis(maltolato) oxovanadium (IV). Can J Physiol Pharmacol 71:263–269.

TEM

Why wait for it to circulate? Read your own copy of

TRENDS IN
ENDOCRINOLOGY
AND METABOLISM

If you share **TEM** with others at work, you know that what goes around doesn't always come around. And if it does, articles are often dated or sometimes clipped.

Why miss a single issue, or wait weeks to read one? By starting your personal subscription, you'll be guaranteed to read each and every issue.

TRENDS IN ENDOCRINOLOGY AND METABOLISM's short reviews on "hot" topics can make a difference in your research and practice. Commissioned researchers and clinicians report on advances in endocrinology and its related areas.

Use the business reply cards in this issue to order your personal subscription today—or call Elsevier Customer Service at:

(212)633-3950 or FAX (212)633-3990.

© 1997, Elsevier Science Inc., 1043-2760/97/$17.00 PII S1043-2760(96)00262-7