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Vanadium Salts as Insulin Substitutes: Mechanisms of Action, a Scientific and Therapeutic Tool in Diabetes Mellitus Research

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ABSTRACT: Vanadium and its compounds exhibit a wide variety of insulin-like effects. In this review, these effects are discussed with respect to the treatment of type I and type II diabetes in animal models, *in vitro* actions, antineoplastic role, treatment of IDDM and NIDDM patients, toxicity, and the possible mechanism(s) involved. Newly established CytPTK plays a major role in the bioresponses of vanadium. It has a molecular weight of approximately 53 kDa and is active in the presence of Co^{2+} rather than Mn^{2+}. Among the protein-tyrosine kinase blockers, staurosporine is found to be a potent inhibitor of CytPTK but a poor inhibitor of InsRTK. Vanadium inhibits PTPase activity, and this in turn enhances the activity of protein tyrosine kinases. Our data show that inhibition of PTPase and protein tyrosine kinase activation has a major role in the therapeutic efficacy of vanadium in treating diabetes mellitus.

KEY WORDS: vanadium, peroxovanadium, cytosolic protein tyrosine kinase, insulin-receptor tyrosine kinase, protein phosphotyrosine phosphatases, insulin, diabetes mellitus.

I. INTRODUCTION

Glucose homeostasis depends on the balance between its formation by the liver and its utilization by major insulin-dependent tissues. These include liver, fat, and muscle, and insulin-independent tissues, as well as brain and kidney. The glucose bal-

Abbreviations: BMI, body mass index; **Bt_2cAMP,** dibutyryl cyclic adenosine monophosphate; **HbA_{IC},** glycosylated hemoglobin; **IDDM,** insulin-dependent diabetes mellitus (type 1); **IRS-1,** insulin receptor substrate-1; **InsRTK,** insulin receptor tyrosine kinase; **MAP-kinase,** mitogen-activated protein kinase; **NIDDM,** non-insulin-dependent diabetes mellitus (type II); **PEPCK,** phosphoenolpyruvate carboxykinase; **PTPase,** phosphotyrosine phosphatase; **pV,** pervanadate or peroxovanadate; **$p90^{rsk}$,** 90-kDa ribosomal S6 kinase; **$p70^{S6k}$,** 70-kDa ribosomal S6 kinase; **STZ,** streptozotocin; **CytPTK,** cytosolic protein-tyrosine kinase.

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ance is regulated by several hormones, of which only insulin possesses hypoglycemic properties. While the primary effect of insulin is glucose homeostasis, it also regulates a number of other cellular events, such as ion and amino acid transport, lipid metabolism, gene transcription, protein synthesis and degradation, and DNA synthesis.[1] The biological importance of insulin is most obvious in diabetes.

In type I insulin-dependent diabetes mellitus (IDDM), hyperglycemia results primarily from severe insulin deficiency caused by destruction of the pancreatic β cells. This condition mostly affects teenagers. Currently, the only possible treatment is exogenous insulin, which does not produce a well-controlled normoglycemia and is associated with increased episodes of severe hypoglycemia, the major fear of diabetic patients, with its possible deleterious cerebral impact.[2] The second category, type II, is the noninsulin-dependent diabetes mellitus (NIDDM), a heterogeneous disorder affecting about 5 to 7% of the world population.[3] NIDDM is associated with insulin resistance and defective glucose recognition by the β-cells, leading to a blunted insulin response to glucose.[4] Treatment of NIDDM hinges on dietary measures, backed up by sulfonylureas, biguanides, and insulin. However, because this approach is not completely satisfactory in a relatively large proportion of patients,[5] new therapeutic avenues are being explored to develop oral hypoglycemic drugs that enhance insulin secretion and sensitivity at their target sites.

Today, widespread effort is being made to search for new hypoglycemic agents with high potency and few or no side effects. Troglitazone, aglitazone, pioglitazone,[6] vanadium, and its compounds[7-9] exemplify agents that increase sensitivity to insulin and/or mimic its activation. In this review, the potential therapeutic applications of vanadium and its compounds are discussed.

II. CHEMISTRY OF VANADIUM

Nils Sefstrom, a Swedish chemist, first discovered the new element in 1830, and named it after Vanadis, the Norse Goddess of Beauty, Youth, and Luster. Vanadium is a transition element usually found in relatively low concentrations. The Earth's crust contains approximately 0.02%. It is obtained as a byproduct from the processing of the ores of other metals. Vanadium is also found in the specialized blood cells of sea squirts in a high concentration, about 0.15 *M*, in the form of tunichrome, which is thought to play an important role in cell metabolism.[10]

The chemistry of vanadium is extremely complex because of its multiple oxidation states (+3, +4, and +5), hydrolysis, and polymerization. Thus, vanadate is a very labile system that can interact with potential ligands, such as nitrogen bases and –OH groups, to give mixtures that are labile and difficult to isolate.[11]

III. BIOLOGICAL IMPORTANCE OF VANADIUM

Vanadium has been recognized as an essential nutritional requirement in higher animals. It seems to be a trace element required for normal growth and development, also being necessary for the growth and survival of mammalian cell culture.[12] The intracellular concentration of vanadium in higher animals varies between 0.02 and 1 μ*M*.[13]

In 1979, *in vitro* vanadate solutions were shown to possess insulin-like effects on

glucose metabolism in rat diaphragms.[14] Subsequently, Shechter and Karlish[15] and Dubyak and Kleinzeller[16] reported on the insulin-mimetic actions of vanadate on hexose uptake and glucose metabolism in rat adipocytes. A new era was opened in 1985, when Heyliger and co-workers[17] found that oral administration of sodium orthovanadate to streptozotocin (STZ)-treated diabetic rats normalized the elevated blood glucose and depressed cardiac performance. Ever since, there has been growing interest in the potential biological use of vanadium, with special reference to the treatment of diabetes.

A. *In Vivo* Insulin-Mimetic Actions of Vanadate on Diabetes

Both types of diabetes, I and II, can be reproduced in animals. Injection of STZ into rats specifically destroys pancreatic islet β-cells and within 1 to 3 d produces insulin deficiency and hyperglycemia (type 1 model). The genetically diabetic C57BL/KSJ-db/db, ob/ob, and fa/fa Zucker rats are good models of NIDDM (type II diabetes), lacking insulin and being obese.[18,19] Thus, useful animal models are available for exploring the insulin-mimetic influence of vanadium.

1. Effect of Vanadium on Insulin-Dependent Diabetes (Type 1)

Vanadium (oxidation state, +5) added to the drinking water of STZ-induced diabetic rats normalizes blood glucose (Figure 1) and improves cardiac performance with partial improvement of body weight gain.[17,20–32] The major insulin-like effect of vanadate is enhanced glucose transport in a variety of tissues, adipocytes,[14,15] skeletal muscle,[33] brain,[34] and liver,[20,29] although it also activates glycolysis and glycogenesis, and depresses glycogenolysis and gluconeogenesis.

Similarly, oral administration of vanadyl (oxidation state, +4)[25] or organic vanadyl (+4) complexes[35,36] produce marked glucose lowering effects in STZ-diabetic rats. This insulin-synergic effect of vanadate may correct the abnormal expression of genes coding for key enzymes of glucose metabolism in the liver of STZ-diabetic rats.[37] Liver mRNA levels of glycolytic enzymes, glucokinase, and L-type pyruvate kinase, which are lowered in diabetic rats, were restored by vanadate treatment. In addition, impaired glycogen synthase, glycogen phosphorylase activity, and glycogen content in liver were returned to normal,[22,27,38] and the elevated levels of phosphoenolpyruvate carboxykinase (PEPCK) and the glucose transporter in liver (GLUT2) and muscle (GLUT4) were also reversed.[28,29] These observations clearly are consonant with vanadate normalizing blood glucose levels.

Vanadate, a potent inhibitor of PTPases, inhibits hepatic PTPase *in vivo* in STZ-diabetic rats.[39,40] Short-term (for 3 d) administration of vanadate in STZ and BB rats resulted in a reduction of PTPase activity in the particulate fraction,[39] which was associated with a change in blood glucose toward normal.

At the same time there was a marginal gain in body weight, which suggested that the long-term effects seen with chronic vanadyl treatment might be related to the functional improvement of β cells. Indeed, Brichard et al.[21] reported significant elevation of pancreatic insulin levels in STZ-diabetic rats after 60 d of vanadate treatment. A restored and/or preserved insulin secretion may in fact be essential for maintained reversal of the diabetic state.[41]

These prolonged euglycemic effects of vanadium may be due to the long half-lives of vanadium in different tissues[42]

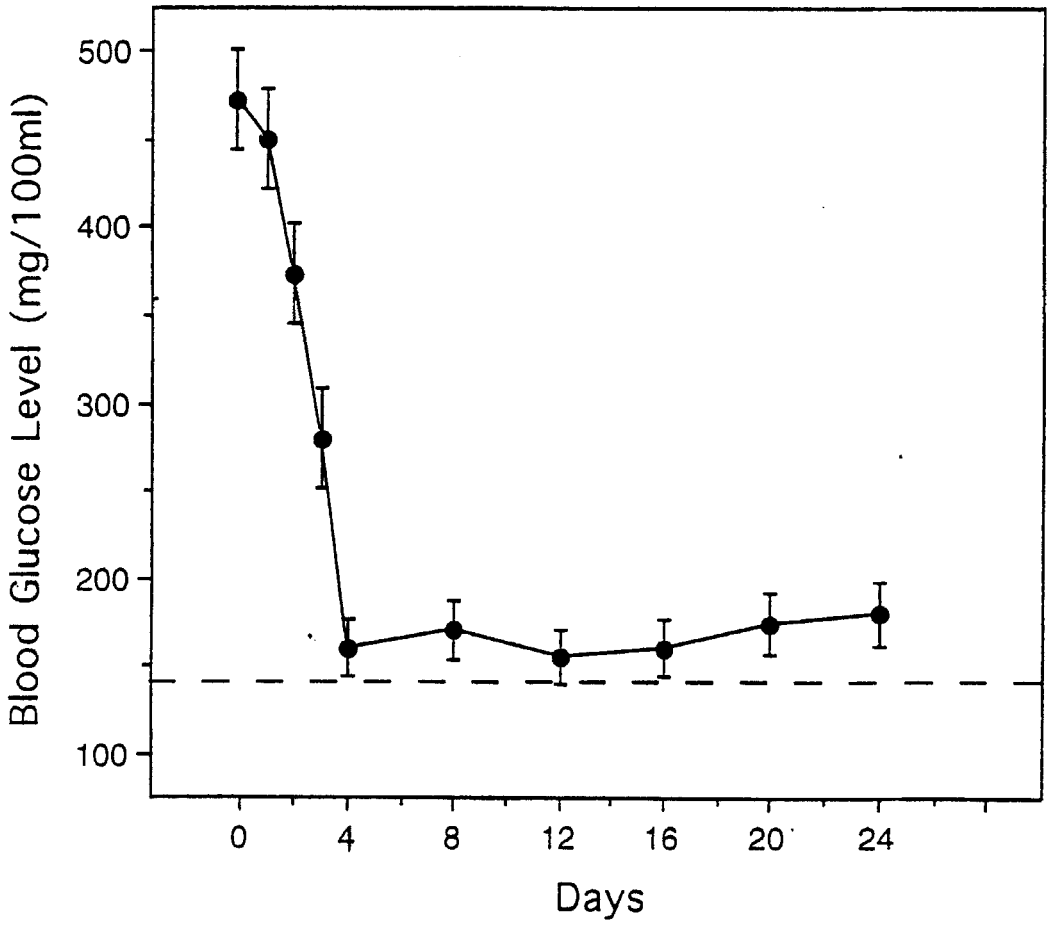

FIGURE 1. Oral administration of vanadate normalizes blood glucose in STZ-diabetic rats. Rats received 0.2 mg $NaVO_3$/ml in the drinking water; blood glucose levels dropped to near-normal values within a few days and retained the low glucose level as long as $NaVO_3$ was included in the drinking water. (From Meyerovitch, I., Farfel, Z., Sack, J., and Schechter, V. 1987. *J. Biol. Chem.*, **262:** 6658–6662. With permission.)

and, more critically, may depend on improved pancreatic function in long-term treatment.[32]

In addition to its influence on glucose homeostasis and carbohydrate metabolism, vanadium also plays a major role in lipid metabolism, restoring the altered plasma lipids and lipoprotein profile in STZ diabetes to near-normal levels.[25,43,44] In 1994, Brichard et al.[45] reported on the lipogenic effects of vanadate. mRNA levels of acetyl CoA carboxylase and fatty acid synthase activities are raised again in liver but not in adipose tissue,[45] and the decreased activity of glucose-6-phosphate dehydrogenase and malic enzyme reverts to normal.[46] Vanadate also inhibits ketoacidosis in type 1 diabetes. The activity of the key regulatory enzyme of liver ketogenesis, 3-hydroxy-3-methylglutamyl-CoA synthase, which is overexpressed in liver mitochondria, is suppressed by vanadate, and normal ketogenesis is restored.[37]

Thus, vanadate treatment in insulin-dependent diabetes tends to normalize plasma glucose homeostasis, carbohydrate and lipid metabolism, and ketogenesis. The insulin-like effect may be due to activation of pancreatic functions or to the inhibition of PTPase activity.

2. Effect of Vanadium on Noninsulin-Dependent Diabetes (Type II)

Resistance of target tissues to the actions of insulin is the other major cause of glucose intolerance. Oral administration of vanadate to genetically obese mice/rats improves glucose homeostasis, tolerance to glucose load, and markedly decreases plasma insulin levels.[47-51] Euglycemic hyperinsulinimetic clamping in vanadate-treated fa/fa rats largely enhanced body glucose disposal, mediated by insulin.[52]

Insulin initiates its pleiotropic effects on cell metabolism by binding to its specific cell-surface receptors.[52] Type II diabetes is characterized by a blunted response to insulin at the receptor and/or postreceptor level.[53] Neither insulin receptor number nor affinity to insulin, and insulin receptor tyrosine kinase (IRTK) activity were altered much in vanadate-treated NIDDM rodents, suggesting that vanadate action is distal to the insulin receptors.[49,51,54] The vanadate-induced improvement in glucose transport activity in the muscle of fa/fa rats is due to enhanced translocation efficiency and/or increased GLUT-4 intrinsic activity with no alteration in transporter number.[54]

In contrast to fa/fa rats, Pugazhenthi et al.[55,56] reported that in vanadate-treated Zucker rats the number of insulin receptors in the liver increased by 119%. In addition, activities of lipogenic enzymes such as glucose-6-phosphate dehydrogenase, malic enzyme, acetyl CoA carboxylase, and ATP-citrate lyase are corrected, and plasma insulin, cholesterol, and triacylglycerol levels are decreased.[50,57,58] These observations point to the actions of vanadate being effected through insulin-mediated pathways.

Vanadate treatment of ob/ob mouse normalizes blood glucose, but the increased expression of PEPCK mRNA is not accompanied by increased expression of glyceraldehyde-3-phosphosphate dehydrogenase mRNA. Thus, it is unlikely that vanadate exerts its insulinomimetic effect at the mRNA level of PEPCK.[59] Its hypoglycemic action may be through an increase of glucose uptake in the peripheral tissues, such as fat and muscle.

However, the glucose homeostases were observed uniformly in NIDDM rats/mice treated with vanadate. The observed difference on insulin receptor may be due to:

1. Variation in the concentration of vanadium administration (0.25 to 0.80 mg/ml in the drinking water)
2. The variation in the duration of treatment
3. Severity of hyperglycemia, hyperinsulinemia, and difference in rat species

B. *In Vivo* Effect of Vanadium Complexes

Even though vanadate and vanadyl (+4) are orally active, they are still not well absorbed. Therefore, organically chelated vanadium complexes have been used. Several of those appeared to be effective insulinmimetic agents at lower doses and reduced the gastrointestinal side effects of vanadium treatment as well.[60] Administration of *bis*(cysteine, amide-*N*-octyl) oxovanadium (iv),[35] *bis*(maltoalto)oxovanadium (iv),[61] and *bis*(picolinato) oxovanadium[62] to STZ-diabetic animals reduced the elevated levels of plasma glucose and lipids, thereby maintaining euglycemia and improving heart function. Yale et al.[63] developed stable peroxovanadium compounds that contain an oxo ligand, one or two peroxo anions, and an ancillary ligand in the inner coordination sphere of vanadium. These peroxovanadium compounds, which are also ~100-fold more active than vanadate in *in vitro* systems, markedly decreased plasma glucose in both

normal and diabetic BB rats.[63] Administration of these peroxovanadium compounds to insulin-deprived BB rats over a period of 3 d significantly reduced blood glucose levels and ketone bodies.[63] Administration of peroxovanadium to STZ rats lowered the blood glucose level as quickly and effectivly as did insulin.[64] Mechanistically peroxovanadium compounds differ from vanadate. They facilitate their insulin-like effects by activating the insulin-receptor (see below).

C. *In Vitro* Insulin-Like Effects of Vanadium

Vanadate (+5), as a structural analog of phosphate, can permeate into the cell interior through the phosphate or the anion carrier system.[65] Theoretically, vanadium can combine with cellular molecules to form various complexes such as nicotinamide adenine dinucleotide-vanadate (NAD-V), adenosine diphosphate-vanadate (ADP-V), guanosine diphosphate-vanadate (GDP-V), a protein-vanadate (protein-V) complex, etc.[66] Although the insulinomimetic effects of vanadate (+5) is best correlated with the inhibition of protein phosphotyrosine phosphatases, the possibility of such intracellular complexes being involved as well cannot be ignored.

Isolated, intact cells are the least complex experimental tools for biologists to test the insulin-like effects of vanadium. Vanadate was demonstrated to mimic the actions of insulin in enhancing hexose uptake and glucose metabolism[15,16,67] and in inhibiting lipolysis.[68] This was followed by research in which the insulin-mimicking effects of vanadate were examined in a large variety of insulin-responsive cells and also in the cell free state. It turned out that vanadate mimics almost any known bioeffect of insulin. This includes the key actions of the hormones, such as increased glucose transport in adipose and muscle tissues, increased glucose metabolism, activation of glycogen synthesis, and inhibition of the breakdown of triglycerides to fatty acids. Vanadate also mimicked the so-called secondary actions of insulin, such as an increase in Ca^{2+} influx, inhibition of Ca^{2+}, Mg^{2+}-ATPase in plasma membranes, stimulation of potassium uptake, and elevation of cytosolic pH (reviewed in Reference 7).

The list of hepatic, *in vitro*, and *in vivo* insulin-like effects of vanadate has progressively increased and includes both metabolic and prolonged activities. Vanadate inhibits the release of glucose from isolated perfused rat liver,[69] stimulates the expression of (L-type) pyruvate kinase and glucokinase mRNA in rat hepatocytes,[70] and inhibits fructose-2,6-bisphosphatase activity, thereby activating glycolysis. Expression of PEPCK mRNA, the rate-limiting enzyme of gluconeogenesis, is also inhibited by vanadate in hepatoma cells.[71] Vanadate potentially inhibits glucose-6-phosphatase activity and therefore arrests gluconeogenesis and glycogenolysis.[72] In isolated perfused rat liver, vanadate and vanadyl (+4) inhibited glucose output and suppressed glucose production by 60%.[69] In muscle, vanadate enhances glucose uptake, and activates glycogen synthesis and glycolysis to a lesser extent than insulin. However, vanadate induces a greater stimulation of lactate and glucose oxidation *in vivo*.[73] The latter may be a secondary effect following the induction of normoglycemia.

D. Effect of Vanadate in Manifesting the Late Effects of Insulin

Several reports have demonstrate that vanadate also mimics the late effects of in-

sulin related to mitogenesis (reviewed in Reference 7). Thus, in several cell types, vanadate activates the MAP-kinase cascade, the 90-kDa ribosomal S6 kinase ($p90^{rsk}$), and the 70-kDa ribosomal-S6-kinase, as does insulin.[74–79] A still open question is whether these late effects are secondary to InsRTK activation. Short preincubation of cells with vanadate does not activate the insulin receptor, but peroxovanadium (pV) does.[80,81] Recently, we found that certain cells are capable of oxidizing cell-entered vanadate into pV or to a highly related compound (in preparation). This phenomenon occurs in a precipitous fashion following a lag period of 5 to 6 h. Thus, several documented late effects of vanadate may occur subsequent to pV formation and insulin receptor activation (personal communication).

E. *In Vitro* Insulin-Like Effects of Peroxovanadium

Vanadate combined with H_2O_2, a weak insulinomimetic agent,[82–86] showed a strong synergistic effect, and potently activated the insulin receptor and the corresponding biological effects in rat adipocytes.[80,81,87–89] This was due to the formation of pV.[87]

Peroxides of vanadium (peroxovanadium, pervanadate, or pV) are about 100-fold more potent than vanadate in manifesting the biological effects of insulin.[80,88] Yu et al. have reported that pbv(pic) (bisperoxopicolinato oxovanadium) stimulates glucose transport at low concentrations and enhanced insulin-binding capacity to intact rat adipocytes. The latter was due to an apparent increase in receptor affinity.[89] It was suggested that pV, or pbv(pic), inhibits InsRTK-associated PTPase(s) and therefore promotes phosphorylation on tyrosine moieties and its activation.[80,81] These observations show that peroxovanadate and its complex manifest insulin-like effects through insulin-dependent pathways.[80,81,87–89] pV compounds differ from vanadium in being oxidizing agents, relative to glutathione.[90] Although 100-fold more potent than vanadate, this undesirable oxidizing feature should be taken into consideration.

F. Effects of Vanadium on Insulin Receptor-Independent Pathways

Because vanadate is a potent inhibitor of cellular PTPases,[91] it was initially believed that vanadium acts intracellularly by blocking these PTPases, which dephosphorylate the insulin receptor, and therefore activates it in an insulin-independent manner. This may not be the case. Numerous groups have reported no significant increase in the phosphotyrosine content of the insulin receptor of vanadate-treated cells or tissues.[74,75,92–97] This suggested the existence of *alternative* pathways for obtaining the effects of insulin in an insulin receptor-independent fashion. This notion was further substantiated by the finding that the activating effects of vanadate on hexose transport and lipogenesis were not blocked by quercetin. Quercetin blocks the activities of both InsRTK in cell-free experiments and insulin-stimulated hexose uptake and lipogenesis in intact rat adipocytes.[92] Vanadate also stimulated glucose uptake in cultured L6 muscle cells by mechanism(s) independent of protein kinase C and InsRTK activities.[98] A cytosolic protein-tyrosine kinase participates in several of the insulin-like effects of vanadate in rat adipocytes. Shisheva and Shechter found that the 40,000 × g supernatant fraction of rat adipose homogenate contains a soluble protein tyrosine kinase (CytPTK).[95,96] The basic features of this enzyme are summarized in Table 1. Like

InsRTK, CytPTK readily phosphorylates $PolyGlu_4Tyr$, a random copolymer of about 30 kDa. However, CytPTK is distinct from InsRTK; the cytosolic enzyme has an estimated molecular weight of 53 ± 3 kDa, and its activity is supported by Co^{2+} rather than by Mn^{2+}. Unlike InsRTK, CytPTK is resistant to inactivation by *N*-ethylmaleimide. Of the several protein tyrosine kinase inhibitors tested, staurosporine was the most potent inhibitor of CytPTK (IC_{50} = 1 to 3 n*M*), but a poor inhibitor of InsRTK [IC_{50} = 8 μ*M*, Table 1).[95]

Vanadate mimics several insulin-like effects in rat adipocytes via a staurosporine-sensitive CytPTK.[92,95,96] Activation of CytPTK in intact rat adipocytes was highly specific for vanadates. Neither insulin, isoproterenol, dibutyryl cAMP, okadaic acid, hydrogen peroxide, nor the phorbol ester TPA altered CytPTK activity (Figure 2).[95] Because staurosporine is a potent inhibitor of CytPTK, in intact adipocytes, staurosporine selectively inhibited the effect of vanadate but had only a marginal effect on insulin-stimulated lipogenesis (Figure 3). However, the insulin-like effects of vanadate in enhancing hexose uptake and in inhibiting lipolysis were not quenched by staurosporine. Thus, vanadate can facilitate several additional insulin-like effects through mechanism(s) not involving CytPTK activation or activation of the insulin receptor.

G. Activation of CytPTK by Vanadate in a Cell-Free System

The steps linking vanadium pretreatment to the activation of CytPTK at the intact cell level can be significantly elucidated if the activating effect is preserved after cell disintegration. Such a cell-free system has been established.[101] The findings and the conclusions made in these cell-free studies are as follows: vanadate activated CytPTK three- to fivefold; the ED_{50} value was calculated to be 3 ± 0.7 μ*M*. Other agents that activated the cytosolic enzyme were tungstate (ED_{50}, ~15 μ*M*), molybdate (ED_{50}, ~25 μ*M*), and phenylarsineoxide (ED_{50}, ~7.0 μ*M*). Sodium fluoride and vanadyl (+4) did not activate CytPTK. It was concluded that activation of CytPTK by vanadate is secondary to the inhibition of protein phosphotyrosine phosphatases.

Hydrogen peroxide, stoichiometrically oxidized vanadyl to vanadate.[101] Based on these findings, a physiological role for the intracellular vanadium pool has been suggested (illustrated schematically in Figure 4). Any physiological conditions converting vanadyl to vanadate (e.g., H_2O_2 production) will activate CytPTK and consequently CytPTK-dependent bioeffects. A link between vanadate, activation of NADPH oxidase and endogenous activation of tyrosine phosphorylation has been noticed in several studies.[103–105]

TABLE 1
Comparison between CytPTK and InsRTK

	CytPTK	InsRTK
Molecular weight	53 kDa	350–400 kDa
Exogenous substrate	$PolyGlu_4Tyr$	$PolyGlu_4Tyr$
Cofactor	Co^{2+}	Mn^{2+}
Sensitivity to *N*-ethylmaleimide	No effect	Inactivation
Inhibition by staurosporine (IC_{50})	2 n*M*	8 μ*M*

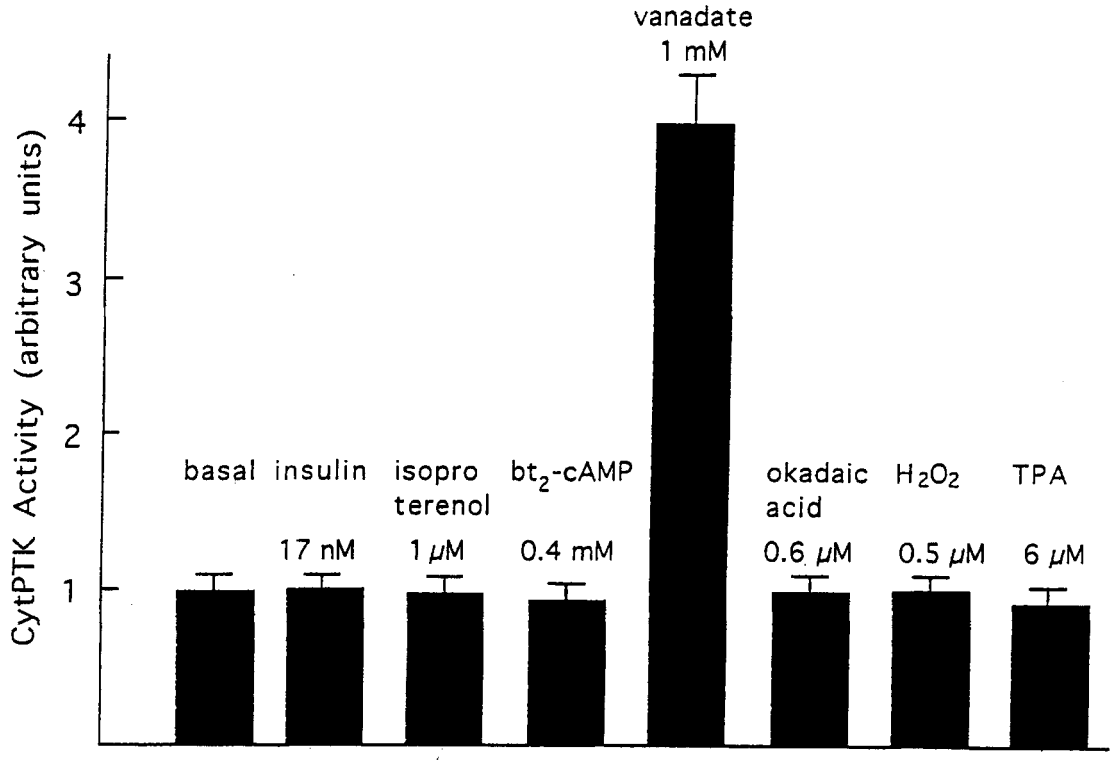

FIGURE 2. Specific activation of cytosolic tyrosine kinase by preincubation with vanadate. (From Shisheva, A. and Shechter, Y. 1992. *FEBS Lett.* **300:** 93–96. With permission.)

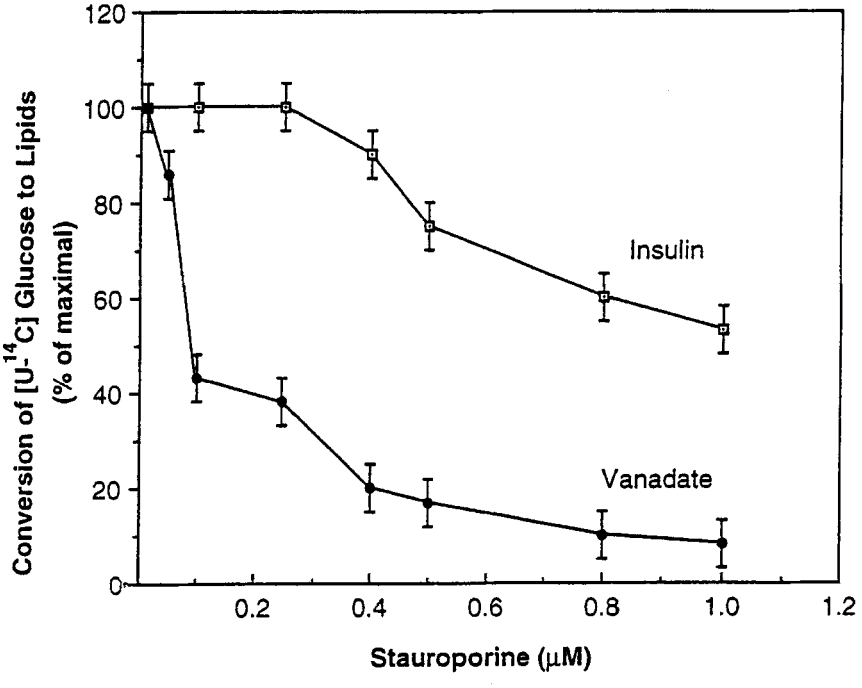

FIGURE 3. Staurosporine inhibits vanadate-stimulated lipogenesis in rat adipocytes. (From Shisheva, A. and Shechter, Y. 1993. *J. Biol. Chem.* **268:** 6463–6469. With permission.)

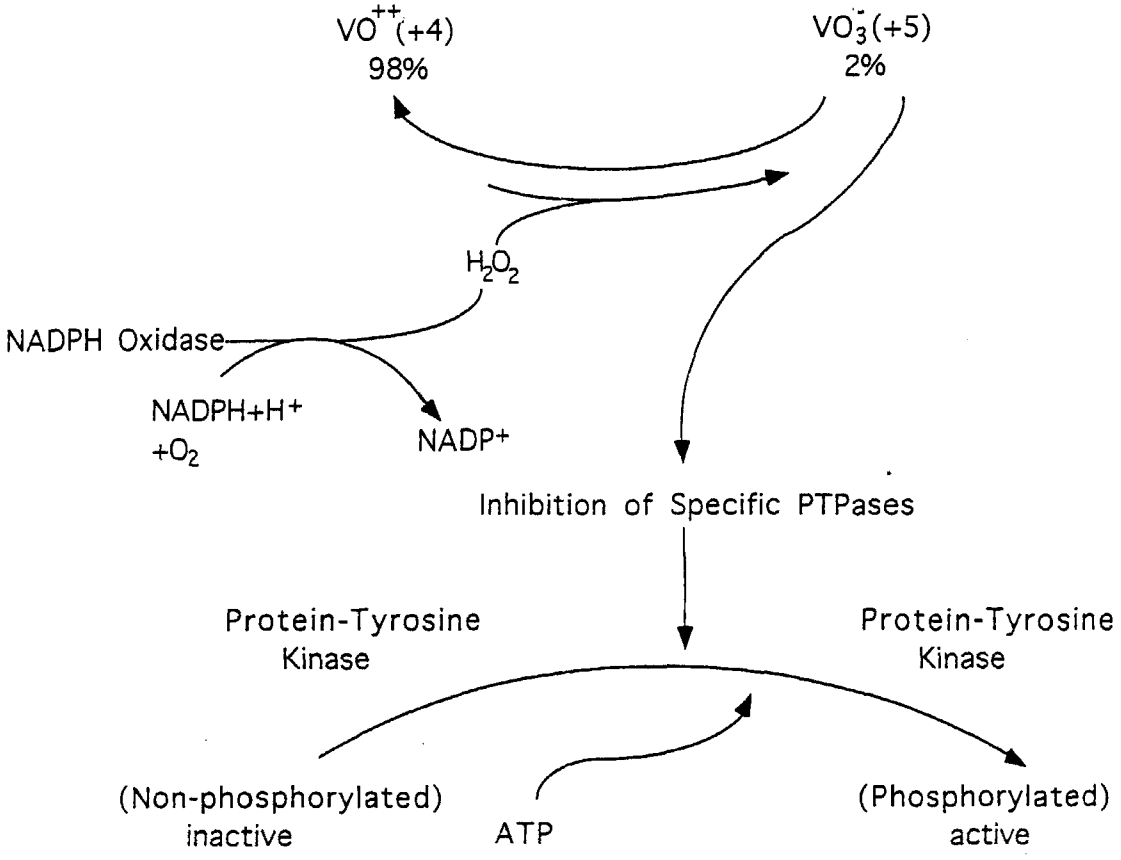

FIGURE 4. Schematic representation of a putative role for the intracellular pool of vanadium in modulating the activity of nonreceptor protein tyrosine-kinases. (From Elberg, G., Li, J., and Shechter, Y. 1994. *J. Biol. Chem.* **269:** 9521–9527. With permission.)

H. Vanadium as an Antioxidant/ Prooxidant

The superoxide radicals produced in the isolated perfused heart by xanthine plus xanthine oxidase were quenched by vanadate treatment.[106] This finding suggested that vanadate can act as a scavenger of oxyradicals and therefore prevent heart dysfunction.[106] These antioxidant features were already observed in low vanadium-treated STZ-rats in an early study.[107] In contrast, vanadium also possesses a prooxidant potential[108–110] and has been shown to produce lipid peroxidation in isolated hepatocytes.[108] This antioxidant/prooxidant role of vanadium requires further clarification. It should be noted, however, that the prooxidant potential of vanadium, observed *in vitro*, may not be in conflict with the antioxidant feature that was produced in the whole-animal model.

I. Vanadium and Antitumor Effects

Although vanadium is best known for its insulin-mimetic effects and for ameliorating diabetes, it also has a role against tumor cells.[111–113] Subcutaneous injections of sodium orthovanadate into mice containing MDAY-D_2 (hematopoetic cell line) tumors resulted in an inhibition of tumor

growth by 85 to 100%.[114] Rijksen et al.[113] demonstrated that vanadate inhibited cell proliferation when added with multiple growth factors. Oral administration of vanadium (0.5 ppm) in drinking water was effective in arresting the development of diethylnitrosamine-induced hepatocarcinogenesis in rats without any toxic manifestation. This chemopreventive action of vanadium could be mediated through inhibition of altered liver cell foci and hepatic nodule growth during the early stages of neoplastic transformation, but not during the promotion period.[115,116] Vanadium has also been shown to promote neoplastic transformation of mouse and hamster fibroblasts[117,118] and to potentiate the transforming activity of c-fps/fes.[119] Vanadate reduced the incidence of mouse colon tumors induced by 1,2-dimethylhydrazine, although thymidine incorporation was increased.[120] The beneficial effects of vanadium concerning cell growth and transformation should be studied further.

J. Vanadium and Human Diabetes

The findings of vanadium salts manifesting their insulinomimetic effects through *alternative* pathways not involving InsRTK activation, and the profound antidiabetic effects observed in insulin-deficient and insulin-resistant diabetic rodents following vanadium therapy encouraged the start of clinical studies. Small doses of vanadium (100 to 125 mg per person per day over a period of 3 weeks) were used. Although 100-fold lower than those used in most animal studies, several beneficial effects were observed. Goldfine et al.,[121] Cohen et al.,[122] and Halberstam et al.[123] have given sodium metavanadate (125 mg/d) and vanadyl sulfate (100 mg/d) orally to both type I and type II diabetic patients for 2 to 3 weeks.

The study of Goldfine et al.[121] included IDDM patients (ages 45 ± 13; BMI, 24.0 ± 1.4 kg/m; HbA_{IC}, 11.1 ± 2.2%) and NIDDM patients (ages 51.8 ± 7.4; BMI, 28.7 ± 3.3 kg/m; HbA_{IC}, 10.9 ± 4.5%) studied before and after oral administration of sodium metavanadate. The experimental duration was 4 weeks, including a 1-week baseline period, 2 weeks of oral vanadate treatment, and 1 week of posttreatment. Patients received vanadate with breakfast (50 mg), lunch (50 mg), and supper (25 mg) over a period of 2 weeks. Following that, two of the five patients (IDDM) showed an improved rate of glucose utilization. More profound effects were obtained with the NIDDM patients, while in all subjects, sensitivity improved during therapy. The improvement of glucose metabolism was due to nonoxidative glucose disposal. In addition to glucose, the level of cholesterol was decreased significantly in both IDDM and NIDDM patients. Further, MAP kinases and S6 kinases were significantly activated in mononuclear cells of vanadate-treated subjects. Mild gastrointestinal intolerance was also observed during the treatment period.[121]

Cohen et al.[122] evaluated the *in vivo* effects of vanadyl sulfate in six NIDDM patients. The NIDDM subjects were initially treated with a placebo (50 mg of lactose) twice daily for 2 weeks, followed by two 50-mg doses of vanadyl sulfate, and then another 2 weeks followed by placebo only. Euglycemic-hyperinsulinemic clamps and oral glucose tolerance tests were performed at the end of each study period. The glycemic index improved significantly, with no alterations in plasma insulin levels.[122] This was attributed to an increased sensitivity of target tissues to insulin and therefore increased glucose uptake, glucose metabolism, glycogen synthesis, and decreased hepatic glucose output. In addition, it is interesting

to observe that most of these beneficial metabolic alterations were sustained for 2 weeks after termination of the treatment.[122,123] The beneficial effects of vanadium on both IDDM and NIDDM may be due to (1) increase in the intrinsic activity or translocation capacity of glucose transporters, (2) improvement of both hepatic and skeletal muscle insulin sensitivity, and (3) accumulation of vanadium in the system.

Further investigation on this potential topic is highly warranted to prove the long-term effectiveness of vanadium and/or its salts without toxicity in diabetes, particularly in NIDDM.

K. Vanadium and Toxicity

Despite the insulin-like and other pharmacological effects of vanadium, the toxicity of this element is of more concern in rodents and humans. The industrial toxicology profile of vanadium has been established.[124,125] Due to its wide use, the biological actions of vanadium are of interest.[126] In general, vanadium compounds enter the body primarily through the lungs, where they are absorbed slowly and excreted mainly in urine.[127] Vanadium fumes induce inflammatory changes in the mucose membranes of the respiratory tract in exposed humans and animals. Inhalation of and exposure to vanadium can cause conjunctivitis, pharyngitis, rhinitis, chronic cough, and tightness of the chest.[126] In medical use, gastrointestinal disorders may also develop.[128]

Studies on the acute oral toxicity of vanadium compounds in rodents showed that both vanadate and vanadyl are moderately toxic, with the severity of the toxic effects increasing as the valency increases.[129] In most studies of the insulin-like effectiveness of vanadium in diabetic rats and mice, vanadium was given orally in the drinking water. Concentrations of 0.2 to 1.1 mg/ml was considered to be toxic, and toxicity increased with increasing concentration of vanadium.[128,129]

Administration of vanadium as metavanadate or orthovanadate causes developmental toxicity in rats[130] and mice,[131] whereas vanadyl ions were embryotoxic and teratogenic in mice when given orally.[132] Hepatotoxicity of vanadium was also proved in isolated perfused hepatocytes.[108,111] In isolated perfused rat livers significant lipid peroxidation was shown. The prooxidant effects of vanadate were found to be mainly responsible for its cytotoxic activity.[111]

Daily food and fluid intake were significantly decreased in vanadium-treated normal and diabetic rats. Although vanadium treatment normalized the blood glucose level, some signs of toxicity were also observed in all of the vanadium-treated animals, as evidenced by decreased body weight gain and increased serum concentrations of urea and creatinine.[133] However, the use of vanadium as an antidiabetic agent is most important. The use of various chelating agents to reduce vanadium toxicity and improve insulin potency are the major recent goals of vanadium research.

IV. SUMMARY AND PERSPECTIVE

Two decades of research on the insulin mimetic effects of vanadium salts yielded interesting basic and clinical implications. From a basic point of view, it appears now that peroxovanadium compounds facilitate their insulin-like effects through activation of the insulin receptor, whereas vanadium salts, both oxidation states (+4) and (+5), utilize *alternative* signaling pathways such as nonreceptor cytosolic and membranal protein-tyrosine kinases. A general overall

message is the illustration of insulin-responsive tissues being equipped with a *backup system* to metabolize glucose (i.e., nonreceptor PTKs) that can be operated when the primary (insulin-dependent), system is faulty. This probably explains the potency of vanadium salts to induce its beneficial antidiabetic effects in diabetic rodents that are poorly responsive to insulin. With respect to a role for vanadium therapy in the future care of diabetes, a major obstacle to be overcome is vanadium toxicity. For example, the LD_{50} for vanadyl sulfate in rat is ~450 mg/kg, whereas the effective antidiabetic dose is ~45 mg/kg. At least two orders of magnitude are required between these two values for further considering its usage as a daily based drug. Several efforts are currently being carried out in this direction. Organic vanadium chelators or binders can significantly potentiate the insulinomimetic features of vanadium and decrease its toxicity. Certainly a better understanding of the backup system(s) involved may eventually lead to the development of more potent and less toxic vanadium substitutes. For example, it currently appears that the vanadium cascade is initiated by inhibiting specific vanadate-sensitive PTPases;[134] this may encourage further identification and characterization of the PTPases involved. Specific cell-permeable inhibitors for these PTPases that will be effective in the nanomolar concentration range may substitute vanadium and assist in the future care of diabetes in human.

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