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Historic perspective and recent developments on the insulin-like actions of vanadium; toward developing vanadium-based drugs for diabetes

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Contents

Abstract

Intensive studies have been carried out during the last two decades, on the insulinomimetic effects of vanadium. Vanadium compounds mimic most of the metabolic effects of insulin on the main tissues of the hormone in vitro. Vanadium therapy induces normoglycemia and improves glucose homeostasis in insulin deficient and insulin resistant diabetic rodents. Improved sensitivity to insulin in liver and muscle tissues of Type II diabetic patients following vanadium therapy was observed as well. The key mechanisms involved are inhibition of protein–phosphotyrosine phosphatases and activation of nonreceptor protein–tyrosine kinases, in an insulin-receptor tyrosine kinase independent fashion. Vanadate activates glucose-metabolism in vitro at a site preceding activation of phosphatidylinositol-3-kinase (PI3-kinase). Regarding inhibition of lipolysis, vanadate (but not insulin) acts at a site downstream to the activation of PI3 kinase. Additional vanadium-dependent mechanism, operating in vivo, is the restoration of glucose-6-phosphate levels in liver, muscle and adipose tissue of hyperglycemic diabetic rats. This is attributed to

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vanadate-dependent inhibition of liver glucose-6-phosphatase, and of nonspecific hexose-6-phosphatases of the diabetic muscle and adipose tissues. Initial clinical studies were already performed. Several beneficial effects were documented. The potential usage of vanadium in the future care of diabetes in human, however, depends on manipulations that would elevate the insulinomimetic efficacy of vanadium without increasing its toxicity. Organically chelated vanadium compounds, in particular, the L-isomer of Glu(γ) monohydroxamate (L-Glu(γ)HXM) are active in potentiating the capacity of free vanadium to activate glucose metabolism, in vitro and in diabetic rats in vivo. L-Glu(γ)HXM differs from other vanadium ligands in being an amino acid derivative that permeates into peripheral tissues through the amino acid transport system. In rat adipocytes, L-Glu(γ)HXM itself activates partially glucose metabolism, by permeating into cell interior, associating with the minute quantity of intracellular vanadium, and turning it into an insulinomimetic active species. L-Glu(γ)HXM, associates with the vanadyl (+4) cation, and the vanadate (+5) anion, at neutral pH with nearly the same binding affinity. Both these oxidation states of vanadium are insulinomimetic. The therapeutical potency of L-Glu(γ)HXM·vanadium complexes is actively studied. Preliminary results on this issue are to be presented.

Keywords: Insulin-like actions of vanadium; Vanadium-based drugs; Diabetes

1. Synopsis

A new era of biomedical/diabetic research was initiated with the discovery of insulin in 1922 [1]. Daily injections of insulin to insulin-deficient patients lowered their high level of blood glucose to normal values and interrupted an otherwise fatal metabolic disorder [1].

Insulin is the mainstay of the treatment of virtually all Type I (insulin-dependent) and many Type II (insulin-independent) diabetic patients. Diabetic patients need to receive insulin via subcutaneous injections, as oral administration of insulin is ineffective in mammals. Insulin stimulates the uptake of glucose, fatty acids and amino acids from the blood circulation for further storage or utilization. Glucose is stored as glycogen in the liver and muscle tissue, fatty acids as triglycerides in the adipose tissue, and amino acids as proteins in the muscle tissue. Insulin also inhibits the action of other hormones that trigger the breakdown of glycogen, lipids and proteins into glucose, fatty acids and amino acids, respectively (reviewed in Ref. [2]).

2. The need for insulin substitutes

Since the discovery of insulin, research has demonstrated the uncontested value of insulin in treating diabetes. Nevertheless, researchers, particularly in the last three decades have been searching for insulin-substitutes to assist in the therapy of the disease. This is particularly valid for Type II (non-insulin)-dependent diabetes mellitus, which accounts for about 90% of diabetic patients [3]. In these patients, the pancreas continues to produce insulin, yet patients remain hyperglycemic as muscle, liver and adipose tissues fail to respond efficiently to insulin. This phenomenon, known as insulin resistance, is a major contributing factor in hyperglycemia in Type II diabetic patients.

3. Search for insulin substitutes

The successful development of methods for preparation of insulin responsive cells in the 1970s (i.e. rat adipocytes, Ref. [4]) facilitated the investigation of the mechanism of insulin action, as well as the identification of several agents that mimic the actions of insulin. Several proteins (trypsin, lectins and antibodies to the insulin receptor) and numerous low molecular weight substances such as H_2O_2, zinc, manganese ions and oubain, activate glucose uptake and glucose metabolism in rat adipocytes (reviewed in Ref. [5]). However, the application of this in vitro gained knowledge to the whole animal model where diabetes is to be controlled, was found impractical for most of these agents. None of them combined the necessary features of absorption, stability in body fluids, low index of toxicity, and specificity in mimicking the bioresponses of insulin. Vanadium compounds may be the exception, provided certain limitations are overcome in future investigations.

4. Vanadium

Vanadium, element number 23 of atomic-weight 50.94, is present in plant and animal cells at concentrations as low as 10–20 nM. This transition metal is relatively abundant ($\sim$0.02%) in nature. Vanadium has an extremely complex chemistry. It can readily change its oxidation state and take an anionic or cationic form. Under physiological conditions it exists predominantly in the anionic form $H_2VO_4^-$, oxidation state (+5), or in the +4 oxidation state as the vanadyl (+4) cation (VO^{2+}). The anionic form resembles phosphate to some extent, while the vanadyl cation resembles Mg^{2+} (reviewed in Ref. [6]).

As with other metal ions in biological systems, vanadium is primarily complexed in vivo. It readily associates with proteins such as transferrin, albumin and hemoglobin, as well as with low molecular weight compounds such as glutathione [7]. It is believed to be

nutritionally essential in mammals, but its specific biological role in humans is still to be elucidated. The total body pool of vanadium in humans is ca. 100–200 μg [8]. The importance of vanadium for growth and development of goats, rats, mice and chicks has been demonstrated [9].

5. Historical link between vanadium salts and human diabetes

In the 19th century, the use of vanadium in humans was recommended in cases of pathological states such as malnutrition, anemia, tuberculosis, and diabetes (reviewed in Ref. [9]). Lyonnet and Martin observed in 1899 that diabetic patients treated with sodium vanadate excreted less glucose in their urine [10]. Understandably, however, that for several decades following the discovery of insulin some 23 years later, insulin substitutes were not considered as potential therapeutic agents.

6. Renewal of interest in vanadium

In the years 1975–1980, a renewed interest in vanadium arose among biochemists and cell biologists. This has been attributed to the efficacy of vanadate (oxidation state +5) to inhibit phosphohydrolases at micromolar quantities (reviewed in Ref. [11,12]). An example for such an enzyme is Na^+, K^+-ATPase [13]. This enzyme facilitates the maintenance of the proper ionic balance by pumping potassium ions into mammalian cells and sodium ions out, utilizing the energy-rich adenosine-triphosphate (ATP). The efficacy of vanadate in inhibiting Na^+, K^+-ATPase is historically linked to the future finding of the insulinomimetic actions of vanadium salts.

6.1. Vanadium compounds mimic insulin

In 1980, the mechanism of insulin action was still largely obscure [14]. A common approach was to apply substances that mimic the actions of insulin in target cells. Oubain and deprivation of K^+ ions, namely conditions that inhibit the activity of Na^+, K^+-ATPase were known to activate glucose-transport and glucose oxidation in rat adipocytes [15]. In this context, the capacity of vanadium salts to mimic insulin was studied as well. In 1980, vanadium salts were demonstrated to mimic the actions of insulin on hexose uptake and glucose metabolism in rat adipocytes [16,17]. Both externally added vanadate (+5) and vanadyl (+4) ions were effective in this respect [16]. Further studies soon revealed that the insulinomimetic actions of vanadium were not related to the inhibition of Na^+, K^+-ATPase, but rather to another mechanism. In fact, unlike its action in cell-free system, vanadium was found incapable of inhibiting Na^+, K^+-ATPase in intact rat adipocytes [16,17]. It was believed at the time that cell-entered vanadium (+5) is efficiently reduced to vanadium (+4) by reduced glutathione [11]. Vanadyl (+4) is an ineffective inhibitor of Na^+, K^+-ATPase [18]. More recent studies discredited this dogma. Reduced GSH was found an ineffectual reductant of vanadium (+5) to vanadium (+4) at physiological pH values [19]. The inability of vanadium to arrest Na^+, K^+-ATPase activity in the intact cell may therefore be attributed to intracellular binding of entered vanadium (+5) or to insufficient concentration to interact with the intracellularly located 'low-ATP' binding domain of Na^+K^+ATPase [20].

6.2. In vitro studies

In the following years (1980–1987) the insulin-like actions of vanadium salts were examined in a large variety of insulin-responsive cells and tissues. It turned out that vanadium salts mimic most of the known rapid (metabolic) effects of insulin. Vanadium salts activate hexose transport in adipose and muscle tissues though at relatively high concentrations ($ED_{50} = 100–200$ μM, Ref. [17]). It activates glycogenesis and inhibits glycogenolysis in liver and diaphragm [6]. In rat adipocytes it stimulates glucose oxidation and lipogenesis and inhibits isoproterenol-mediated lipolysis [21]. Hepatic gluconeogenesis is suppressed by lowering phosphoenolpyruvate carboxykinase (PEPCK)-mRNA levels [22] and by inhibiting the activity of glucose-6-phosphatase (Glc-6-Pase, Ref. [23]), an event that can also be accounted for by the ability of vanadate to arrest glycogen breakdown. Glycolysis in liver is enhanced due to the inhibitory action of vanadate on fructose 2,6-bisphosphatase [24] and the metaloxide's action on the induction of L-type pyruvate kinase gene [25]. In summary, vanadate inhibits several key metabolic enzyme systems in liver, muscle and adipose tissues, all of which act collectively towards utilizing or storing cell-entered glucose, as well as blocking the actions of hormones that oppose insulin action [26].

6.3. Insulin-signaling past and present status

In the seventies, little was known about insulin signaling. It was shown that insulin associates with cell-surface receptor sites of about 300 000 Da, composed of four disulfide-linked subunits. Following insulin binding, hexose uptake was observed to be markedly enhanced within minutes, mediated by facilitated diffusion. The glucose-transport system was proven to be a separate entity unrelated to the insulin receptor (reviewed in Refs. [5,14]). As newly entered-

glucose was metabolized and stored, it was further assumed that insulin activates those metabolic enzyme systems that participate in utilizing and storing glucose. Since glucagon or isoproterenol-dependent breakdown of glycogen and fat was arrested, insulin was assumed to inhibit the enzymic systems participating in hydrolyzing glycogen and fat. The activity of some of those enzymes is modulated by phosphorylation of ser/thr moieties, whereas the activity of others is modulated by dephosphorylation of phosphoserine (pser) and phosphothreonine (pthr) moieties [5]. It was conceptually difficult to understand (as it is still today) the phenomenal efficacy of insulin to modulate and synchronize simultaneously several endogenous events, some of which are dependent on elevation others on a decrease in the extent of ser/thr phosphorylation of key metabolic enzymes and proteins.

Major advances have since been made in this direction. Insulin action on glucose uptake in muscle and fat results from a cascade of signaling events emanating from the insulin receptor and culminating in translocation of the major insulin responsive glucose transporter GLUT-4 from intracellular vesicles to the plasma membranes [27,28]. The insulin-receptor is an insulin-activated protein–tyrosine kinase. Insulin binding to this cell surface transmembrane receptor leads to the autophosphorylation of three tyrosine moieties in the receptor β-subunit [29]. Such autophosphorylation activates the intrinsic tyrosine kinase activity, resulting in tyrosine phosphorylation of several cytosolic docking proteins called insulin-receptor substrates (IRS_s-Ref. [30,31]). The tyrosyl phosphorylated IRS_s are recognized by various effector molecules, including the regulatory subunit of phosphoatidylinositol 3-kinase (PI3-kinase) via the src homology 2(SH2) domain [31,32]. Docking of PI3-kinase activates this enzyme, a prerequisite for insulin action on glucose transport, glycogen synthase, protein synthesis, antilipolysis and inhibition of gluconeogenesis in the liver; the latter activity is regulated by insulin-evoked suppression of PEPCK gene expression (Ref. [33], reviewed in Ref. [34]). An insulin activated serine/threonine kinase named Akt or PKB, discovered and characterized in the last decade [35], is believed to participate in insulin-evoked glucose transport in fat and muscle. Its location in the insulin-dependent cascade is considered to be downstream to PI3-kinase activation [34].

As mentioned above, the actions of insulin in inhibiting glucagon-evoked glycogenolysis in liver, or isoproterenol-mediated lipolysis in adipose tissue, requires a step of phosphoserine/phosphothreonine dephosphorylation. The linkage between insulin-evoked tyrosine phosphorylation and pser/pthr dephosphorylation, i.e. activation of protein-phosphatase 1) must await further elucidation.

6.4. Vanadium facilitates insulin-like actions in an insulin-receptor tyrosine-kinase independent fashion

The finding of endogenous tyrosyl phosphorylation as an early and essential step in the insulin-dependent cascade mechanism first suggested that vanadium, like insulin, activates the insulin-receptor tyrosine kinase (InsRTK). Although in vanadate-treated cells the InsRTK is not significantly activated (i.e. Ref. [36]), doubts remained because activation of 1–3% of the total receptor sites in insulin-responsive cells is sufficient to activate maximally the biological actions of insulin [37]. That InsRTK does not participate in the insulin-like actions of vanadate was convincingly demonstrated when quercetin, a natural bioflavonoid compound, was found to be a blocker of InsRTK-catalyzed transphosphorylation of exogenous substrates (IC_{50} = 1–2 μM, Ref. [38]). In intact adipocytes, quercetin inhibited the actions of insulin in activating glucose transport, glucose oxidation, lipogenesis, and glycogenesis, but did not inhibit at all the very same effects triggered by vanadate [38]. The antilipolytic actions of either insulin or vanadate were not antagonized by quercetin. These findings strongly suggested that InsRTK is not involved and another tyrosine kinase participates in manifesting the insulin-like actions of vanadate on glucose uptake and metabolism. The linkage between vanadate-evoked tyrosyl phosphorylation and the manifestation of its metabolic actions has gained credence in 1996 and 1997 by the usage of wortmannin. This powerful inhibitor of PI3-kinase, an enzyme whose activation requires an early step of tyrosyl phosphorylation [39], blocks all metabolic actions of insulin in rat adipocytes, including the antilipolytic action of the hormone [33]. Regarding vanadate, worthmannin arrested the activating action of vanadate on glucose uptake, glucose oxidation, lipogenesis and glycogenesis, but failed to block the antilipolytic action of vanadate [40,41]. The antilipolytic action of vanadate is therefore independent of PI3-kinase activation and appears to interfere at a very distal site of the lipolytic cascade [40].

6.5. Nonreceptor-cytosolic protein tyrosine kinase

Phosphotyrosine in cells comprises less than 0.1% of the combined amount of phosphoserine and phosphothreonine. Nevertheless, the high-speed supernatant of all mammalian cells contains appreciable amounts of cytosolic-nonreceptor protein tyrosine kinase activity (cytPTK reviewed in Ref. [42]). The physiological role of this enzymic activity is still speculative (subsequent paragraphs). Our studies revealed that spleen, thymus, smooth muscle, lung and kidney of rat are relatively rich in cytPTK activity whereas heart and skeletal muscle are relatively poor. Cytosolic PTK comprises 11–26% of the

total cellular activity while the rest is of membranal origin. Tyrosyl phosphorylation requires the presence of divalent anions such as Co^{2+}, Mn^{2+} and Mg^{2+} and ATP is the best phosphate donor [43]. As with receptor-PTKs, most cytosolic-PTK undergoes reactivation by self-phosphorylation on tyrosine moieties [42]. Staurosporine is a powerful inhibitory marker of cytPTK (IC_{50} value of 1–20 nM) and in general a poor inhibitor of membranal PTKs ($IC_{50} = 1$–40 μM Ref. [43]). With such abundance of cytPTK activity found in mammalian cells, it is somewhat surprising that basal phosphotyrosyl level is negligible. A possible explanation for that is the relatively high cellular activity of protein–phosphotyrosine phosphatases (PTPases) in mammalian cells [44,45]. Such activity appears to prevent cytPTKs from undergoing spontaneous reactivation by self phosphorylation on tyrosyl moieties [43]. This situation may be altered if cellular PTPase activity is reduced, or partly inhibited, by the presence of vanadium (subsequent paragraphs).

6.6. A cytosolic vanadate-activated protein–tyrosine kinase

A cytosolic protein tyrosine kinase that is activated by vanadate in a cell-free system and in rat-adipocytes has been identified and partially purified from the 40 000 g supernatant of rat adipose cells [46,47]. This water-soluble cytosolic-PTK (apparent M.W. = 53 kDa) differs from the InsRTK in many respects, including its sensitivity to inactivation by *N*-ethylmaleimide and susceptibility to inhibition by staurosporine ($K_i = 1$–2 nM, Ref. [46,47]). Studies in a cell-free system, comprising exclusively of the cytosolic (40 000 g) supernatant fraction, revealed that cytPTK is activated by vanadate (+5) 4–6-fold, with an $ED_{50} = 2.0 \pm 0.2$ μM). CytPTK is not activated by vanadyl (+4) ions [48]. Vanadate inhibits adipose–cytosolic-PTPases at the same range of concentration ($IC_{50} = 4 \pm 1$ μM), suggesting, though not proving that cytPTK activation is secondary to cytosolic PTPase inhibition by this metalooxide.

In the intact-adipose cell, staurosporine blocks the activating effects of vanadate on lipogenesis, glucose oxidation, and glycogenesis with $IC_{50} = 0.1$–0.3 μM [41,47]. Insulin-stimulated bioeffects are not inhibited by staurosporine at this low range of concentrations. Low staurosporine concentrations also had no effect on vanadate-stimulated hexose uptake nor on vanadate's antilipolytic action. We concluded that CytPTK participates in the activating effects of vanadate on glucose metabolism but not in the activation of hexose transport, nor in the antilipolytic action of vanadate.

6.7. A putative physiological role for the intracellular vanadium pool in mammals

Although we have focused on insulin-like effects in adipocytes produced by exogeneously added vanadium, the accumulated data also suggest a putative physiological role for the minute quantities of the intracellularly located vanadium. The intracellular concentration of vanadium in human tissues is 0.2–0.8 μM [49], mostly in the chelated vanadium (+4) form [11,12]. The high activity of CytPTK in the mammalian cytosolic compartment [43] raises the question whether it constitutes a reservoir for responding to physiological needs not directly controlled by external stimuli. Within the cytosolic compartment, vanadate, but not vanadyl (+4), activates cytPTK [43]. At neutral pH vanadyl is readily oxidized to vanadate by one equivalent of hydrogen peroxide [48]. The efficiency of this reaction is very high, and vanadyl should efficiently compete with GSH for endogenously formed H_2O_2. Any physiological condition that activates NADPH oxidase and leads to the formation of H_2O_2 is expected to oxidize a fraction of the endogenous vanadyl pool to vanadate, which in turn will inhibit vanadate-sensitive PTPases. This would result in a corresponding increase in the steady state of phosphorylation and activation of the cytosolic protein tyrosine kinases. That vanadyl can be converted to vanadate via an NADPH-oxidative pathway has been demonstrated previously and a link between vanadate, NADPH, and activation of tyrosine phosphorylation in cells has been frequently observed ([48] and reviewed in Ref. [50]).

6.8. A membrane-bound non-receptor-PTK is involved in some actions of vanadium

In addition to the cytosolic nonreceptor PTK identified and partially characterized in 1993 [47], a second vanadium-activated non-receptor PTK activity was identified in 1997 [51]. The latter is a non-glycosylated 55 kDa protein, exclusively located in the plasma membrane (membPTK). Vanadate promotes its autophosphorylation and activates it 7–9-fold in a cell-free experimental system with ED_{50} value of 17 ± 2 μM. Of the many PTK blockers screened, membPTK is inhibited only by staurosporine ($IC_{50} = 60 \pm 5$ nM). In intact adipocytes, staurosporine antagonized vanadate-induced hexose uptake ($IC_{50} = 6.0 \pm 0.3$ μM) and reversed the antilipolytic effect of vanadate ($IC_{50} = 5.0 \pm 0.4$ μM, Ref. [51]).

Following vanadium treatment, the autophosphorylated P55 protein is immunoprecipitated by antibodies both to phosphotyrosine and to PI3-kinase (α-P85), thereby linking PI3-kinase with tyrosine-phosphorylated membPTK. Thus, membPTK might participate in the

activation of PI3-kinase and in propagating the effects of vanadate not involving the CytPTK.

Memb-PTK is activated by vanadate (+5) and more so by vanadyl ions ($ED_{50} = 45 \pm 2$ nM), provided that membrane-PTPases are dissolved and released with Triton X-100 [51]. Although this last cell-free observation may not necessarily reflect the status of the intact cell, several additional studies support the contention that both vanadium (+4) and vanadium (+5) are insulinomimetically active and operate by separate pathways. Vanadyl (+4) is active at the level of the plasma-membrane and is responsible for the facilitation of glucose uptake, and possibly for inhibiting lipolysis whereas vanadate (+5) operates exclusively in the cytosolic compartment in enhancing glucose and fat metabolism.

7. Vanadium therapy: studies in insulin-deficient rats

A widely used rodent model representing Type I diabetes is the streptozocin-treated-rat (STZ-rat). Injection of streptozocin destroys most pancreatic β-cells thus producing insulin-deficient, hyperglycemic rats. Unfortunately, chronic hyperglycemia does not lead to any increase in glucose utilization. On the contrary, it leads to abnormal expression of key enzymes involved in carbohydrate metabolism and to a further decrease in the capacity to dispose of circulating glucose (reviewed in Ref. [2,3,6]).

The inclusion of 0.2 mg ml^{-1} sodium-metavanadate ($NaVO_3$) in the drinking water of STZ-rats normalized circulating glucose levels within 2–5 days of treatment [52,53]. Stable normoglycemia was retained as long as $NaVO_3$ was supplied. Studies carried out in several laboratories have demonstrated dramatic improvements in insulin responsive tissues following 2–3 weeks of stable normoglycemia following vanadium therapy [52–57,62,63,73–75]. In the liver, the elevated insulin binding capacity was fully normalized [53] and key metabolic enzymes and the level of glycogen were restored. Glycogen level was restored from 40 to 109% relative to healthy rats, glucokinase from 0 to 65%, and the level of dephosphorylated (active) 6-phosphofructo-2-kinase from 20 to 122% [54]. In muscle, the decreased level of glucose-6-phosphate, the impaired activity of glycogen synthase, and the reduction in glycogen reserves were all returned to normal values [55,56].

Phlorizin, an inhibitor of renal tubular reabsorption of glucose, is capable of correcting hyperglycemia in diabetic rats [57]. It is devoid, however, of any known insulin (or vanadium) effects on metabolizing glucose at the tissue level [57]. A comparison between vanadium- and phlorizin-induced therapy can therefore suggest whether the induction of prolonged-stable normoglycemia per se following vanadium therapy can account for all the ameliorating actions of this metalooxide in the STZ-diabetic rat. Induction of normoglycemia with phlorizin restored the various abnormalities of the diabetic liver, but not those of the diabetic muscle [56,58]. Thus, regarding the diabetic muscle, vanadium therapy appears to facilitate additional beneficial effects not accounted for by the induction of normoglycemia by vanadate.

Despite these improvements, vanadium therapy is not expected to replace insulin in insulin-deficient diabetic models. For example, STZ-rats are largely catabolic. Daily weight-gain can be fully corrected by insulin therapy, but only partially (20–30% of insulin effect) by vanadium therapy [53]. The reason for the catabolic nature of the untreated STZ-rat model is likely to be an increased rate in muscle protein degradation, a process that can be arrested by insulin, but not by vanadate [59].

7.1. Vanadium therapy in insulin-independent diabetic rodents

Four genetically well studied rodent models for Type II diabetes are ob/ob mice, db/db mice, BB rats and fa/fa rats. These hyperglycemic and hyperinsulinemic rodents share a common denominator of having a blunted response to insulin at the receptor and post-receptor levels. Exogenously administered insulin is incapable of reducing blood glucose levels or ameliorating any of the defects associated with utilization of glucose. Various tissues derived from those rodents fail to metabolize glucose appreciably in response to insulin [60].

Oral vanadate therapy improved glucose homeostasis in ob/ob and db/db mice. Therapy led to marked and sustained decrease in glycemia and insulinemia [61]. Tolerance to glucose load has been improved as well [62] and the depletion of pancreatic insulin stores, which often takes place upon aging of these diabetic rodents, has been minimized [60]. Of the many parameters studied, these improvements correlated best with an increased capability of peripheral tissues (especially muscle) to respond to insulin [64]. The mechanism of this sensitizing action of vanadium is still obscure. Therapy did not increase the number of insulin receptor sites, nor the affinity of insulin for receptor binding. The capacity of the insulin receptor to undergo autophosphorylation or to phosphorylate endogenous substrates remained unaltered as well. Also, the content of GLUT4 glucose transporters in muscle remained unmodified [63]. Whatever the mechanism is, glycemia has been improved in these rodents following 8–12 days of vanadium therapy. Moreover, upon cessation of therapy, circulating glucose levels remained low for additional 2 weeks before hyperglycemia reoccurred [61]. Thus, once the sensitizing effect has been established, it appears to remain stable over prolonged periods after therapy has ended.

7.2. Additional vanadium actions in diabetic rodents in vivo

Based on studies during the last two decades, the antidiabetic actions of vanadium can be attributed to (a) inhibition of protein phosphotyrosine phosphatases and activation of nonreceptor protein tyrosine kinases; (b) inhibition of several other enzymes that participate in glucose and fat metabolism, such as fructose 2.6 bisphosphatase, glucose-6-phosphatase, and hormone sensitive lipase; and (c) an increase or a decrease in mRNA levels of L-Type pyruvate kinase and phosphoenolpyruvate carboxykinase, respectively (previous paragraphs).

Recent findings suggested an additional mechanism that operates in vivo and might contribute significantly to the impaired glucose homeostasis in diabetic rodents. The level of glucose-6-phosphate (G-6-P) was found to be reduced by 23, 50 and 42% in adipose, liver and muscle, respectively, in hyperglycemic STZ-rats relative to healthy control rats [58]. Vanadium therapy fully restored G-6-P level in all three insulin-responsive peripheral tissues [58]. The decrease in liver G-6-P was not surprising, because glucokinase activity in STZ-rats is essentially lacking [54]. The decrease in G-6-P in adipose and muscle tissue was unexpected. Unlike glucokinase, chronic hyperglycemia does not significantly decrease the level and activities of hexokinase I and II in muscle and adipose tissues [64].

Further studies in this direction have revealed that rat muscle and adipose homogenates possess a considerable amount of hexose-6-phosphate-dephosphorylating activity, which is inhibited by micromolar concentrations of vanadate (i.e. Ref. [41]). The restoration of G-6-P level in vanadium-treated STZ-rats can therefore be due to the inhibition of glucose-6-pase in liver and those nonspecific hexose-6-phosphatases present in muscle and adipose tissues. G-6-P is the metabolite involved in the stimulation of lipogenic enzyme gene expression in response to glucose [65]. It is also known to be an allosteric activator of glycogen-synthase. It binds to glycogen synthase and induces a conformational change that favors dephosphorylation and activation of this enzyme by protein–phosphatase-1G [66].

8. Organovanadium complexes

Several clinical studies have already been performed in humans with vanadium salts, using low doses of vanadium (2 mg kg^{-1} day^{-1}) to avoid toxicity. Although 10–20-fold lower than those used in most animal studies, even these doses of vanadium yielded several beneficial effects [67–71]. Any manipulation that elevates the insulinomimetic efficacy of vanadium without increasing its toxicity is of significance for the future care of diabetes in humans.

Various organically chelated vanadium compounds are more potent than free vanadium salts in facilitating insulin-like effects in vitro and in vivo ([72–74], reviewed in Ref. [75]). These organically chelated vanadium compounds appear to have in common the property of increasing permeation into tissues by passive-diffusion and, correspondingly, increased insulinomimetic efficacy, with little or no decrease in indices of toxicity of this metalooxide. We have therefore searched for another class of vanadium synergizing ligands. Screening was directed toward finding vanadium ligands that (a) have negligible indices of toxicity; (b) are capable of crossing plasma-membranes (alone or complexed to vanadium) through naturally occurring transport systems; and (c) do not necessarily increase vanadium permeation into tissues but are capable of converting already present intracellular vanadium into an insulinomimetically active species.

Amino acid hydroxamates were found appropriate. Within this family, the L-isomer of glutamic acid γ-monohydroxamate, potentiated vanadium (+5) to activate glucose uptake and glucose metabolism in rat adipocytes in vitro (by 4–5-fold) and lowered blood glucose levels in hyperglycemic rats in vivo (by 5–7-fold, Ref. [76]). A molar ratio of two L-Glu(γ)HXM molecules to one vanadium (+5) was most effective. L-Glu(γ)HXM partially activated lipogenesis in rat adipose cells in the absence of exogenous vanadium. This effect was not manifested by D-Glu(γ)HXM. This and other related studies suggested that L-Glu(γ)HXM, following its entrance through the amino-acid transport system, is capable of converting the minute quantity of intracellular vanadium (+4) into an active insulinomimetic compound [76].

8.1. Criteria for ligands that potentiate the insulin-like potency of vanadium

As L-Glu(γ)HXM was most effective in vitro and in vivo we considered it the optimal vanadium ligand and searched for features underlying its unusual synergistic potency. A comparison was made with other vanadium ligands that are ineffective or less effective in synergizing the insulinomimetic capacity of vanadium. To this end, cell-free experimental systems were developed to determine ligand affinities toward vanadium (+4 and +5) at physiological pH values. Experiments were also conducted in a rat adipocytic experimental system in which the intracellular pool of vanadium had been enriched about 25-fold. Also, studies were conducted to see whether the geometry of an optimal vanadium complex can be determined by chemophysical measurements. L-Glu(γ)HXM and L-Asp(β)(HXM) were found to share the following three distinct criteria, whereas less active

ligands possess only one or two of them: (a) they associate with vanadium (+5) at pH 7.2 within a narrow range of association constant (K^{eff}) of 1.3–1.9×10^2 M^{-1}; (b) they have nearly the same binding affinity for the vanadyl (+4) cation and the vanadate (+5) anion at physiological pH values; (c) they form an intense ultraviolet-absorbing complex upon associating with vanadium (+4) at 1–3 molar stoichiometry. Vanadium ligands lacking any of these three defined criteria synergize less effectively with vanadium to activate glucose metabolism both in vivo and in vitro [76,77].

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References

[1] F.G. Banting, C.H. Best, J.B. Collip, W.R. Campbell, A.A. Fletcher, Can. Med. Assoc. J. 12 (1922) 141.
[2] C.R. Kahn, Y. Shechter, in: A.G. Gilman, T.W. Rall, A.S. Nies, P. Taylor (Eds.), Goodman and Gilman Textbook of Pharmacology, eighth ed (Chapter 61), Pergamon Press, Oxford, 1989, p. 1463.
[3] S.N. Davis, D.K. Graner, in: J.G. Hardman, L.E. Limbird, R.W. Ruddon, A.G. Gilman (Eds.), Goodman and Gilman, The Pharmacological Basis of Therapeutics, ninth ed, Pergamon Press, New York, 1996, p. 1487.
[4] M. Rodbell, J. Biol. Chem. 239 (1964) 375.
[5] M.P. Czech, Ann. Rev. Biochem. 46 (1977) 359.
[6] S.M. Brichard, J.C. Henquin, Trends Pharmacol. Sci. 16 (1995) 265.
[7] N.D. Chasteen, E.M. Lord, H.J. Thompson, J.K. Grady, Biochim. Biophys. Acta 884 (1986) 84.
[8] B.R. Nechay, L.B. Naninga, P.S.E. Nechay, R.L. Post, J.J. Grantham, I.G. Macara, L.F. Kubena, D.P. Timothy, F.H. Nielson, Fed. Proc. 45 (1986) 123.
[9] Y. Shechter, A. Shisheva, Endevour 17 (1993) 27.
[10] B.M. Lyonett, E. Martin, La Press Medicale 1 (1899) 191.
[11] T.J.B. Simons, Nature (London) 281 (1979) 337.
[12] I.G. Macara, Trends Biochem. Sci. 5 (1980) 92.
[13] L.C. Cantley, L. Josephson, R. Warner, M. Yanigasawa, C. Lechene, G. Guidotti, J. Biol. Chem. 252 (1977) 7421.
[14] M.P. Czech, Diabetes 29 (1980) 399.
[15] J.N. Fain, Endocrinology 83 (1968) 540.
[16] Y. Shechter, S.J.D. Karlish, Nature 284 (1980) 556.
[17] G.R. Dubyak, A. Klienzeller, J. Biol. Chem. 255 (1980) 5306.
[18] L.C. Cantley, P. Aisen, J. Biol. Chem. 252 (1977) 7421.
[19] J. Li, G. Elberg, D.C. Crans, Y. Shechter, Biochemistry 35 (1996) 8315.
[20] L.C. Cantley, L.G. Cantley, L. Josephson, J. Biol. Chem. 253 (1979) 7361.
[21] H. Degani, M. Gochin, S.J.D. Karlish, Y. Shechter, Biochemistry 20 (1981) 5795.
[22] F. Bosch, M. Hatzoglov, E.A. Park, R.W. Hanson, J. Biol. Chem. 265 (1990) 13677.
[23] J. Singh, R.C. Nordlie, R.A. Jorgenson, Biochim. Biophys. Acta 678 (1981) 477.
[24] M.H. Rider, R. Bartrons, L. Hue, Eur. J. Biochem. 190 (1990) 53.
[25] M. Miralpeix, J.F. Decaux, A. Kahn, R. Bartrons, Diabetes 40 (1991) 462.
[26] Y. Shechter, Diabetes 39 (1990) 1.
[27] M.G. Myers, M.F. White, Ann. Rev. Pharmacol. Toxicol. 36 (1996) 615.
[28] G.D. Holman, M. Kasuga, Diabetologia 40 (1997) 991.
[29] O.M. Rosen, Science 237 (1987) 1452.
[30] R.A. Wilden, K. Siddle, E. Haring, J.-M. Backer, M.F. White, C.R. Kahn, J. Biol. Chem. 267 (1992) 13719.
[31] B.L. Lavan, G.S. Lienhard, J. Biol. Chem. 268 (1993) 5921.
[32] X.J. Sun, P. Rothenberg, C.R. Kahn, J.M. Backer, E. Araki, P.A. Wilden, D.A. Cahill, B.J. Goldstein, M.F. White, Nature 352 (1991) 73.
[33] T. Okada, Y. Kawano, T. Sakakibara, O. Hazeki, M. Ui, J. Biol. Chem. 269 (1994) 3568.
[34] B.B. Kahn, Cell 92 (1998) 593.
[35] S.K. Moule, G.I. Welsh, N.J. Edgell, E.J. Foulstone, C.G. Proud, R.M. Denton, J. Biol. Chem. 272 (1997) 7713.
[36] G.I. Fantus, S. Kadota, G. Deragon, B. Foster, B.I. Posner, Biochemistry 28 (1987) 8864.
[37] Y. Shechter, The Receptors, vol. II, Academic Press, 1985, p. 221.
[38] A. Shisheva, Y. Shechter, Biochemistry 31 (1992) 8059.
[39] M.G. Myers, J.M. Backer, X.Y. Sun, S. Shoelson, P. Hu, J. Schlessinger, M. Yoakim, B. Schaffhausen, M.F. White, Proc. Natl. Acad. Sci. USA 89 (1992) 10350.
[40] J. Li, G. Elberg, N. Sekar, Z. He, Y. Shechter, Endocrinology 138 (1997) 2274.
[41] N. Sekar, J. Li, Z. He, D. Gefel, Y. Shechter, Endocrinology 140 (1999) 1125.
[42] A.K. Srivastava, Int. J. Biochem. 22 (1990) 1229.
[43] G. Elberg, J. Li, A. Leibovitch, Y. Shechter, Biochim. Biophys. Acta. 1296 (1995) 299.
[44] H. Charbonneau, N.K. Tonks, Ann. Rev. Cell. Biol. 8 (1992) 463.
[45] S.K. Brown-Shimer, K. Johnson, D. Hill, A. Bruskin, Cancer Res. 52 (1992) 478.
[46] A. Shisheva, Y. Shechter, FEBS Lett. 300 (1992) 93.
[47] A. Shisheva, Y. Shechter, J. Biol. Chem. 268 (1993) 6463.
[48] G. Elberg, J. Li, Y. Shechter, J. Biol. Chem. 269 (1994) 9521.
[49] V. Sitprija, S.E. Ong, in: J.O. Nriagu (Ed.), Vanadium and Metabolic Problems in Vanadium in the Environment (Part 2: Health Effects), Wiley, 1998, p. 91.
[50] Y. Shechter, J. Li, J. Meyerovitch, D. Gefel, R. Bruck, G. Elberg, D.S. Miller, A. Shisheva, Mol. Cell. Biochem. 153 (1995) 39.
[51] G. Elberg, Z. He, J. Li, N. Sekar, Y. Shechter, Diabetes 46 (1997) 1680.
[52] C.E. Heyliger, A.G. Tahiliani, J.H. McNeill, Science 227 (1985) 1474.
[53] J. Meyerovitch, Z. Farfel, J. Sack, Y. Shechter, J. Biol. Chem. 262 (1987) 6658.
[54] J. Gil, M. Miralpeix, J. Carreras, R. Bartrons, J. Biol. Chem. 263 (1988) 1868.
[55] N. Venkatesan, A. Avidan, M.B. Davidson, Diabetes 40 (1991) 492.
[56] L.D. Rossetty, M.R. Laughlin, J. Clin. Invest. 84 (1989) 892.
[57] L.D. Rossetty, G.I. Smith, D.P. Shulman, R.A. DeFronzo, J. Clin. Invest. 79 (1987) 1510.
[58] Q. Sun, N. Sekar, I. Goldwaser, E. Gershonov, M. Fridkin, Y. Shechter, Am. J. Physiol. Endocrinol. Metab. 279 (2000) E403.

[59] A.S. Clark, J.M. Fagan, W.E. Mitch, Biochem. J. 232 (1985) 273.
[60] S.M. Brichard, C.J. Bailey, J.C. Henquin, Diabetes 39 (1990) 1326.
[61] J. Meyerovitch, P. Rothenberg, Y. Shechter, S. Bonner-Weir, C.R. Kahn, J. Clin. Invest. 87 (1991) 1286.
[62] S.M. Brichard, A.M. Pottier, J.C. Henquin, Endocrinology 125 (1989) 2510.
[63] S.M. Brichard, F. Assimacopoulos-Jeannet, B. Jeanrenaud, Endocrinology 131 (1992) 311.
[64] R. Burcelin, M. Eddouks, J. Maury, J. Kande, R. Assan, J. Girrard, Diabetologia 38 (1995) 283.
[65] F. Foufelle, B. Gouhot, J.P. Pegorier, D. Perdereau, J. Girard, P. Ferre, J. Biol. Chem. 267 (1992) 20543.
[66] C. Villar-Palasi, Biochim. Biophys. Acta 1244 (1991) 203.
[67] N. Cohen, M. Halberstam, P. Shlimovich, C.J. Chang, H. Shamoon, L.D. Rossetty, J. Clin. Invest. 95 (1995) 2501.
[68] A.B. Goldfine, D.C. Simonson, F. Filli, M.E. Patti, C.R. Kahn, Mol. Cell. Biochem. 153 (1995) 217.
[69] M. Halberstam, N. Cohen, P. Shlimovich, L.D. Rossetty, H. Shamoon, Diabetes 45 (1996) 659.
[70] G. Boden, X. Chem, J. Ruiz, G. Van Rassum, S. Turco, Metabolism 45 (1996) 1130.
[71] K. Cusi, S. Cukier, R.A. DeFronzo, M. Torres, F.M. Puchulo, J.C. Pereira-Redondo, J. Clin. Endocrinol. Metabol. 86 (2001) 1410.
[72] Y. Shechter, A. Shisheva, A.C. Lazar, J. Libman, A. Shanzer, Biochemistry 31 (1992) 2063.
[73] H. Watanabe, M. Nakai, K. Komazawa, H. Sakurai, J. Med. Chem. 37 (1994) 876.
[74] H. Sakurai, K. Fujii, H. Watanabe, H. Tamura, Biochem. Biophys. Res. Commun. 214 (1995) 1095.
[75] K.H. Thompson, J.H. McNeill, C. Orvig, Chem. Rev. 99 (1999) 2561.
[76] I. Goldwaser, J. Li, E. Gershonov, M. Armoni, E. Karnieli, M. Fridkin, Y. Shechter, J. Biol. Chem. 274 (1999) 26617.
[77] I. Goldwaser, S. Qian, E. Gershonov, M. Fridkin, Y. Shechter, Molec. Pharmacol. 58 (2000) 738.