Antidiabetic Action of Vanadyl in Rats Independent of In Vivo Insulin-Receptor Kinase Activity

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The effects of oral vanadyl sulfate administration for 9–12 days on carbohydrate and lipid metabolism in the basal state and on glucose dynamics during submaximal hyperinsulinemic clamps were investigated in nondiabetic and streptozocin-induced diabetic rats. Decreases in growth rate and water and food consumption were the only significant alterations noted in control animals receiving vanadyl. Administration of vanadyl to diabetic rats resulted in weight loss; a significant decrease in plasma glucose, triglyceride, and cholesterol levels; and decreases in food and water intake, without a concomitant change in plasma insulin concentrations. Vanadyl treatment did not modify either peripheral glucose utilization or hepatic glucose production in control rats during submaximal insulin clamps. In contrast, vanadyl therapy increased insulin-induced glucose utilization significantly and had a small but nonsignificant effect on insulin-mediated suppression of glucose production in diabetic rats. The tyrosine kinase activity of liver- and muscle-derived insulin receptors from diabetic rats that underwent clamp study, which reflected the in vivo phosphorylation state of insulin receptor, was not altered by vanadyl treatment. In conclusion, these results show that augmentation of peripheral glucose utilization is the major determinant of the antidiabetic action of vanadyl and support the notion that the action of vanadyl is independent of insulin-receptor kinase activity. *Diabetes* 40:492–98, 1991

Both the vanadate^{5+} and vanadyl^{4+} forms of vanadium have been shown to elicit several insulinlike effects (for review, see ref. 1). These include the ability to increase glucose transport and oxidation by rat adipocytes (2,3) and skeletal muscle (4,5), pentose phosphate pathway (6) and glycogen synthase activity in rat adipocytes (7), and glycogen synthesis in the liver and diaphragm (8) and the ability to inhibit adrenocorticotropin-induced lipolysis in rat adipocytes (9).

Heyliger et al. (10) were the first to show that oral administration of vanadate to streptozocin-induced diabetic (STZ-D) rats normalized their blood glucose concentration. This effect was observed in the absence of increased serum insulin levels, suggesting that vanadate has an insulinlike effect on glucose metabolism in vivo. These observations have subsequently been confirmed in several other studies (11–15). Brichard et al. (16) and Pederson et al. (17) reported that the tolerance of STZ-D rats to oral or intravenous glucose challenge was either normalized or considerably improved by vanadate treatment and that this was not due to restoration of insulin release. However, these studies do not clarify whether the insulin sensitivity of glucose-producing or glucose-utilizing pathways is modified by vanadium treatment.

This study was therefore undertaken to assess the relative importance of enhanced glucose utilization by peripheral tissues versus decreased glucose production in the antidiabetic action of vanadium with the insulin-clamp technique in conjunction with the isotopic measurement of glucose turnover. In addition, the possibility that the vanadium effect is related to insulin-receptor kinase activation in vivo was examined in view of the proposed role for insulin-receptor kinase in mediating insulin action (18,19).

RESEARCH DESIGN AND METHODS

Male Sprague-Dawley rats weighing 230–260 g were obtained from Bantin & Kingman (Fremont, CA) and housed at 21 ± 2°C with 12-h alternating light-dark cycles. One group was injected with STZ (55 mg/kg i.v.) to induce diabetes, whereas another group received only the vehicle (0.1 M citrate, pH 4.5) and served as age-matched controls. Induction of diabetes was confirmed by measuring glucosuria

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(>28 mM) 1 wk later. The control and diabetic groups were then divided into two further subgroups: one group continued to receive plain tap water (untreated control and untreated diabetic), whereas the other group was given drinking water supplemented with 0.5 mg/ml vanadyl sulfate trihydrate (treated control and treated diabetic). The rats were maintained for 9 (control) or 12 (diabetic) days with free access to water and laboratory chow. Vanadyl, rather than vanadate, was chosen because of its lower toxicity (8,20).

Clamp studies. After an overnight fast, rats were anesthetized with Inactin (100 mg/kg i.p.), and through a ventral midline neck incision, the left carotid artery and right jugular vein were catheterized with PE-50 tubing for blood sampling and infusion, respectively. The animals were also given a tracheotomy with a small plastic T-tube connected to low-flow O_2. After obtaining baseline blood and urine samples, a primed (0.2-ml) continuous (0.02-ml/min) infusion of [3-^{3}H]glucose (6 μCi/ml) was started to measure glucose turnover rate (21). A primed continuous infusion of pork insulin (5 mU · kg^{-1} · min^{-1}) was begun simultaneously to acutely raise and maintain the plasma insulin concentrations at submaximal level while infusing somatostatin (1.5 μg · kg^{-1} · min^{-1}) to inhibit endogenous insulin secretion. Plasma glucose concentration was kept constant at 7.8 mM in untreated and vanadyl-treated control groups by determining plasma glucose every 7–8 min and empirically adjusting the infusion rate of a 20% glucose solution. Because the glycemic threshold for counterregulatory hormone release would be expected to be higher in diabetic compared with control rats, there was the risk of stimulating epinephrine release and measuring the opposite effects of epinephrine and insulin on glucose disposal during the clamp study if the diabetic rats were clamped at the euglycemic level (7.8 mM; 22). Moreover, blood glucose concentration itself affects glucose uptake by its mass action independent of insulin (23). Hence, untreated and vanadyl-treated diabetic groups were clamped at the hyperglycemic level (13.3 mM).

Plasma samples for glucose specific activity were obtained at 10-min intervals between 90 and 120 min after the start of the infusion. The rate of appearance of glucose was calculated from the non-steady-state derivation of Steele's equation (24) and is equal to the rate of glucose utilization (R_d) under clamp conditions. The amount of glucose appearing in the urine of the diabetic rats during the clamp period (90–120 min) was subtracted from R_d to obtain corrected R_d. The difference between R_d and the exogenous glucose infusion rate represents the residual endogenous glucose production.

At the end of the clamp, rectus muscle and liver were rapidly dissected, frozen in liquid N_2, and stored at −70°C.

Isolation of insulin receptors. Insulin receptors were purified from untreated and vanadyl-treated diabetic rats that had undergone clamp study with modifications of previously described methods (25,26). Two-gram portions of liver were homogenized in a buffer consisting of 250 mM sucrose, 5 mM EDTA, 1 mg/ml benzamidine, 1 mg/ml bacitracin, 10 mM NaF, 10 mM tetrasodium pyrophosphate, 1 mM molybdate, 100 KIU/ml aprotinin, 1 mM phenylmethylsulfonyl fluoride (PMSF), and 50 mM HEPES (pH 7.4). The homogenate was centrifuged at 10,000 × *g* for 10 min, and the supernatant was decanted and centrifuged at 40,000 × *g* for 45 min. The resulting microsomal pellet was suspended in solubilizing buffer (homogenizing buffer with the omission of sucrose and supplemented with 1.5% Triton X-100), allowed to solubilize for 1 h at 4°C, and centrifuged at 120,000 × *g* for 45 min. The supernatant was then incubated with 1 ml settled wheat-germ agglutinin (WGA)-agarose for 1 h at 4°C. The WGA-agarose was centrifuged at 2000 × *g* for 5 min, and the supernatant was discarded. The WGA-agarose was washed three times with buffer containing 100 mM NaCl, 5 mM EDTA, 100 KIU/ml aprotinin, 10 mM NaF, 10 mM tetrasodium pyrophosphate, 1 mM molybdate, 0.1% Triton X-100, 1 mM PMSF, and 50 mM HEPES (pH 7.4). The insulin receptor was eluted by incubating the WGA-agarose in wash buffer supplemented with 0.3 M *N*-acetyl-D-glucosamine, aliquoted, and stored at −70°C.

One-gram pieces of rectus muscle were fragmented and homogenized in the solubilizing buffer and insulin receptors purified and recovered as described above for liver.

Binding studies and kinase assays. Aliquots of receptor preparations containing 5 μg protein were incubated with 173 pM ^{125}I-labeled insulin at 4°C for 16 h in the absence or presence of various concentrations of unlabeled insulin in 80 μl 50 mM HEPES (pH 7.4) containing 100 mM NaCl, 0.1% Triton X-100, 1 mg/ml bacitracin, and 20 mM $MgCl_2$. Receptor-bound insulin was precipitated with 12.5% polyethylene glycol in the presence of bovine γ-globulin as carrier (27). Nonspecific binding was defined as the radioactivity precipitated in the presence of 1.73 μM unlabeled insulin. For kinase assays, receptor preparations were adjusted to similar receptor concentration by relative binding potency as described by Burant et al. (26).

Individual receptor preparations were diluted with eluting buffer to contain 40 (for muscle) or 60 (for liver) fmol and incubated with either anti-insulin-receptor antibody (final dilution 1:100) or normal human serum (1:50) for 18 h at 4°C. These conditions ensured quantitative precipitation of insulin receptor by the antibody (data not shown; 28). The samples were immunoprecipitated with protein A–agarose and washed three times to remove inhibitors, and the immune complex was resuspended in 40 μl of a buffer containing 100 mM NaCl, 2.5 mM KCl, 1 mM $CaCl_2$, 0.05% Triton X-100, 1 mM molybdate, and 50 mM HEPES (pH 7.4).

After resuspension, 20 μl of a phosphorylation mixture was added to yield final concentrations of 1 mg/ml histone 2B, 5 mM $MnCl_2$, 10 mM $MgCl_2$, 0.5 mM CTP, 1 mM molybdate, 4 μM [γ-^{32}P]ATP (100 Ci/mmol), and 50 mM HEPES (pH 7.4). The reaction was allowed to proceed at 4°C for 5 min and then terminated by the addition of an equal volume of two-fold–concentrated Laemmli's buffer (29) containing 0.2 M dithiothreitol. The samples were then heated at 100°C for 5 min and the supernatants subjected to electrophoresis on 14% acrylamide gels.

After staining, gels were incubated in 0.85 M KOH at 55°C for 2 h to hydrolyze serine and threonine phosphates (30), dehydrated, dried, and submitted to autoradiography with Kodak X-Omat AR film. Radioactivity in the histone band was determined by subtracting the radioactivity present in an equal area of the same lane in which no discrete phosphoproteins were identified. Specific insulin-receptor–mediated phosphorylation of histone was deduced by subtraction of

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histone-associated radioactivity in the presence of normal human serum from that in the presence of antibody.

The effectiveness of molybdate in inhibiting phosphatase activity was assessed as follows. Insulin receptors, isolated in the absence of phosphatase inhibitors, were incubated with 4 μM[γ-^{32}P]ATP in the absence or presence of 1 mM molybdate under conditions similar to those described for histone kinase assay. After 30 min at 4°C, aliquots of the reaction mixture were added to 2 ml 50 mM KH_2PO_4 containing 0.1 g Norit A charcoal. The mixture was vortexed vigorously and the charcoal pelleted by centrifuging at 4°C at 1000 × *g* for 10 min. The percentage of total radioactivity remaining in the charcoal-free supernatant represented the ^{32}P hydrolyzed from ATP (31).

Glucose concentrations in serum and urine were determined by a Beckman glucose analyzer (Irvine, CA). Serum insulin was measured by a double-antibody technique with the kit supplied by ICN (Irvine, CA). Triglyceride, cholesterol, and high-density lipoprotein (HDL) cholesterol concentrations in serum were determined with the kit supplied by Sigma (St. Louis, MO). Serum [3-^{3}H]glucose radioactivity was determined in the supernatants after precipitating protein with perchloric acid (21). Protein content of insulin-receptor preparations was measured by the Bradford dye method (32).

The following items were obtained from the indicated sources: pork insulin and all materials for electrophoresis from Sigma; somatostatin from Bachem (Torrance, CA); WGA-agarose from Vector (Burlingame, CA); protein A–agarose from Repligen (Cambridge, MA); [3-^{3}H]glucose, ^{125}I-insulin, and [γ-^{32}P]ATP from ICN; and Inactin from Byk-Gulden (Konstanz, Germany). All other chemicals were of reagent grade and obtained from standard suppliers.

Statistical analyses were performed with Student's two-tailed *t* test.

RESULTS

Weight gain and food and water intake were reduced in vanadyl-treated control rats compared with untreated controls (Table 1). There was no effect of vanadyl on either the glucose or insulin levels of plasma in control rats. As expected, diabetic rats did not gain weight, and they consumed more food and water than controls; their plasma glucose levels were markedly elevated, whereas plasma insulin concentrations were diminished. Administration of vanadyl to diabetic rats resulted in weight loss, a significant decrease in plasma glucose, and decreases in food and water intake without having any apparent effect on circulating insulin levels. The glucosuria of vanadyl-treated diabetic rats measured at the beginning of the clamp study was markedly reduced: <2.8 mM in treated versus 56 mM in untreated rats.

The effects of vanadyl treatment on lipid metabolism are summarized in Table 2. Vanadyl administration had no effect on plasma triglyceride and total cholesterol levels in control rats. Diabetic rats exhibited significantly elevated levels of triglycerides and total cholesterol compared with controls. Treatment of diabetic rats with vanadyl led to a marked diminution in circulating triglyceride and total cholesterol concentrations. There was no difference in HDL cholesterol levels among the four groups of rats studied.

Clamp studies. The effects of vanadyl treatment on insulin-mediated glucose metabolism are shown in Table 3. The steady-state plasma concentrations of insulin are lower in diabetic rats compared with controls under similar conditions of insulin infusion (5 mU · kg^{-1} · min^{-1}), indicating that the clearance of insulin is elevated in diabetes; similar results have been reported by other workers (33). The well-documented increases in insulin binding to tissues (34) and in the degradation rate of plasma [^{3}H]insulin measured in vivo (35) in the insulin-deficient state could explain this increased metabolic clearance rate of insulin in the diabetic animals. Vanadyl administration did not modify either insulin-stimulated peripheral glucose utilization or insulin-mediated suppression of hepatic glucose production in control rats. In contrast, vanadyl treatment augmented insulin-induced glucose utilization significantly (by 57%) and slightly increased (20%) insulin-mediated suppression of glucose production in diabetic rats.

Insulin binding. The competitive binding curves of ^{125}I-insulin to receptors solubilized and partially purified from the liver and rectus muscle of diabetic rats that underwent clamp studies are presented in Fig. 1. Insulin binding to liver receptors was consistently lower in vanadyl-treated diabetic than untreated diabetic rats (Fig. 1*A*). The average binding capacity of liver receptors was 20.8 ± 0.6 fmol/5 μg protein for untreated diabetic rats compared to 17.3 ± 1.0 fmol/5 μg protein for treated diabetic rats ($P < 0.001$), indicating

TABLE 1
Physiological characteristics of control and diabetic rats with and without vanadyl treatment

	Control		Diabetic	
	−Vanadyl (*n* = 9)	+Vanadyl (*n* = 10)	−Vanadyl (*n* = 9)	+Vanadyl (*n* = 10)
Weight gain (g/day)	7.2 ± 0.8	4.4 ± 0.7*	0.0 ± 0.6†	−1.9 ± 0.6‡
Food intake (g/day)	32.8 ± 1.2	28.3 ± 1.2*	48.5 ± 2.5†	31.5 ± 2.0§
Water intake (ml/day)	48.1 ± 3.1	33.3 ± 1.6*	212.0 ± 10.6†	64.8 ± 3.4§
Plasma glucose (mM)	8.7 ± 0.4	7.9 ± 0.2	26.9 ± 0.5†	15.3 ± 1.9§
Plasma insulin (pM)	318.0 ± 23.7	346.8 ± 10.1	170.2 ± 23.7†	197.4 ± 28.0

Values are means ± SE. Diabetes was induced by a single intravenous injection of streptozocin (55 mg/kg). Vanadyl sulfate in drinking water (0.5 mg/ml) was administered to control rats for 9 days and to diabetic rats for 12 days. Plasma glucose and insulin were determined in the basal postabsorptive state.
*$P < 0.02$, †$P < 0.001$, vs. control rats without vanadyl.
‡$P < 0.05$, §$P < 0.001$, vs. diabetic rats without vanadyl.

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TABLE 2
Effect of vanadyl treatment on plasma lipid metabolism in control and diabetic rats

	Control		Diabetic	
	−Vanadyl (n = 10)	+Vanadyl (n = 8)	−Vanadyl (n = 10)	+Vanadyl (n = 9)
Triglycerides (mg/dl)	54.1 ± 3.5	57.7 ± 6.6	489 ± 134*	82.3 ± 16.7†
Total cholesterol (mM)	1.33 ± 0.10	1.22 ± 0.07	3.02 ± 0.47*	1.89 ± 0.08‡
HDL cholesterol (mM)	0.94 ± 0.08	0.81 ± 0.04	0.99 ± 0.06	1.00 ± 0.07

Values are means ± SE. Plasma was obtained from animals fed ad libitum until death. HDL, high-density lipoprotein.
*$P < 0.005$ vs. control rats with or without vanadyl.
†$P < 0.01$, ‡$P < 0.05$, vs. diabetic rats without vanadyl.

reversal by vanadyl of upregulated insulin binding in diabetic rat liver, as observed in other studies with vanadate (11). However, the binding of muscle-derived insulin receptors was not influenced by vanadyl treatment (7.7 ± 0.5 vs. 7.4 ± 0.3 fmol/5 μg protein; Fig. 1*B*).

Insulin-receptor kinase activity. Because the in vivo phosphorylation state of the insulin receptor was measured in this study, endogenous phosphotyrosine phosphatases had to be inhibited during insulin-receptor preparation and kinase assay. Because vanadate, a potent phosphatase inhibitor (36), could not be used, we tested the feasibility of using molybdate as a phosphatase inhibitor. The amount of ATP hydrolyzed by insulin receptor was 9.9% in the presence and 25.2% in the absence of 1 mM molybdate; background hydrolysis with buffer alone was 4.4%. In addition, Strout et al. (37) reported 90% inhibition of a phosphotyrosine phosphatase from rat liver membranes by molybdate. Accordingly, 1 mM molybdate was included during receptor purification and kinase assay. The ability of insulin receptors derived from either liver or muscle tissue of diabetic rats to phosphorylate the exogenous substrate histone in vitro was not altered by vanadyl treatment (liver: 7.1 ± 0.8 and 7.1 ± 0.8 fmol ^{32}P incorporated into histone in 5 min without and with vanadyl, respectively; skeletal muscle: 2.0 ± 0.6 and 2.5 ± 0.8 fmol ^{32}P incorporated into histone in 5 min without and with vanadyl, respectively; n = 7/group).

DISCUSSION

In this study, the effectiveness of oral vanadyl treatment on various parameters of carbohydrate and lipid metabolism in the basal state and on glucose dynamics during insulin clamp was investigated in nondiabetic and STZ-D rats. In addition, the possibility that alterations in insulin-receptor kinase activity may mediate the antidiabetic action of vanadyl was tested.

Administration of vanadyl to nondiabetic rats resulted in slower growth rate and reduced consumption of food and water without any effect on the plasma levels of glucose, insulin, triglyceride, cholesterol, and HDL cholesterol. Essentially similar results have been reported in other studies (12–15). In two studies (14,15), plasma insulin levels were found to be decreased without any change in plasma glucose, implying increased insulin sensitivity by vanadyl under euglycemic conditions.

As expected, administration of STZ to rats led to hyperglycemia, hypoinsulinemia, hypertriglyceridemia, and hypercholesterolemia. Treatment of diabetic rats with vanadyl decreased their food and water intake and plasma concentrations of glucose, triglyceride, and total cholesterol without any concomitant change in insulin levels, in agreement with other studies (13,15,38). This reduction in serum triglyceride and cholesterol levels is probably a consequence of decreased glucose concentrations in treated diabetic rats, be-

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TABLE 3
Glucose kinetics during clamps in control and diabetic rats with and without vanadyl treatment

	Control		Diabetic	
	−Vanadyl (n = 9)	+Vanadyl (n = 10)	−Vanadyl (n = 9)	+Vanadyl (n = 10)
Steady-state plasma glucose (mM)	7.6 ± 0.2	7.7 ± 0.1	13.7 ± 0.4	13.3 ± 0.4
Steady-state plasma insulin (nM)	1.01 ± 0.09	1.12 ± 0.09	0.71 ± 0.05*	0.74 ± 0.06*
Glucose utilization rate ($mg \cdot kg^{-1} \cdot min^{-1}$)	23.6 ± 1.2	21.8 ± 1.0	18.9 ± 1.6	29.7 ± 1.6†
Glucose infusion rate ($mg \cdot kg^{-1} \cdot min^{-1}$)	18.2 ± 1.0	17.0 ± 1.2	10.0 ± 2.2	22.5 ± 1.6†
Glucose production rate ($mg \cdot kg^{-1} \cdot min^{-1}$)	5.4 ± 0.5	4.8 ± 1.0	8.9 ± 0.9	7.2 ± 1.4

Values are means ± SE.
*$P < 0.01$ vs. control rats with or without vanadyl.
†$P < 0.001$ vs. diabetic rats without vanadyl.

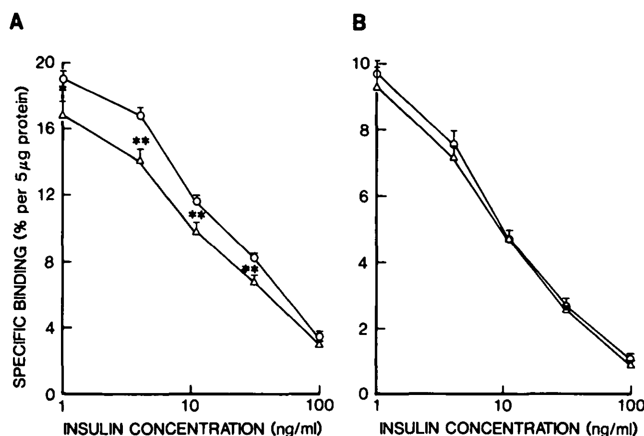

FIG. 1. Insulin binding to receptors partially purified from liver (*A*) and muscle (*B*) tissues of diabetic rats with (△) and without (○) vanadyl treatment. Tracer concentration was 17,850 counts/min (cpm)/tube for liver and 15,130 cpm/tube for muscle. Nonspecific binding in presence of 1.73 μM insulin was 7.69 ± 0.11% for liver and 7.41 ± 0.12% for muscle. *Data points* are means ± SE of 8 animals/group. *$P < 0.05$; **$P < 0.02$.

cause vanadyl treatment had no effect on any of these parameters in control rats. The decrease in body weight caused by vanadyl in diabetic rats in our study is similar to the findings of Brichard et al. (16) and Pugazhenthi and Khandelwal (39). However, the body weight of diabetic rats receiving vanadate therapy was found to be unaffected in other studies (12,13,15). These differences are possibly due to differences in age and severity of diabetes of the animals used. Note that therapy with 0.5 mg/ml vanadyl did not achieve normoglycemia in diabetic rats in this study. Pederson et al. (17) reported that, at 0.5 mg/ml, 71% of the diabetic rats were normoglycemic, whereas higher concentrations of vanadyl (≥0.75 mg/ml) were necessary to obtain 100% normoglycemia. With a different therapeutic regimen (0.8 mg/ml metavanadate for 14 days), Bendayan and Gingras (14) observed that diabetic animals could be divided into two subgroups: one with a stable response (normoglycemia) and another with an unstable response (partial amelioration of hyperglycemia). Treatment with a suboptimal dose of 0.5 mg/ml orthovanadate also resulted only in partial improvement in hyperglycemia (39). Thus, it is not surprising that we observed only partial reversal of hyperglycemia with 0.5-mg/ml vanadyl therapy.

A reduction in daily food intake and an acceleration of kidney glucose excretion could theoretically contribute to the hypoglycemic action of vanadium. However, these mechanisms are unlikely to play a major role for the following reasons. Although vanadyl-treated diabetic rats consumed less food than untreated diabetic rats, their food intake was comparable to that of nondiabetic rats (Table 1). When untreated diabetic rats were pair fed with vanadate-treated diabetic rats, no differences were noted between ad libitum–fed and pair-fed diabetic groups in glucose kinetics during submaximal hyperinsulinemic clamps (13), implying that reduction in food intake per se does not enhance insulin-mediated glucose metabolism in diabetic rats. Furthermore, increases in liver glycogen levels of STZ-D rats subsequent to vanadate treatment have been reported (12,16). Finally, glucosuria is either absent or negligible in vanadium-treated diabetic rats (16; this study).

A major point of interest that emerges from our data is that vanadyl treatment exerts different effects on glucose metabolism in vivo by the liver and peripheral tissues in the diabetic condition. Peripheral glucose utilization induced by submaximal insulin levels was markedly elevated in treated diabetic rats, whereas glucose production (mainly by the liver) was only marginally suppressed in the clamp studies (Table 3). This is consistent with the findings of Rossetti and Laughlin (38) that vanadate treatment of partially pancreatectomized diabetic rats corrects the defect in peripheral insulin-mediated glucose disposal with no effect on residual glucose production. In contrast, Blondel et al. (13) observed a marked decrease in glucose production and a modest increase in glucose utilization under a different set of experimental conditions (rats rendered diabetic by a lower dose of STZ [45 mg/kg] and treated with metavanadate for 20 days). Furthermore, they observed that the glucose production rate in the basal state was normalized in diabetic rats receiving vanadate therapy (13). Vanadium treatment had no effect on either glucose utilization or glucose production during submaximal insulin clamps in nondiabetic rats (13; Table 3).

The lowering of plasma glucose in the absence of an increase in circulating insulin levels suggests that vanadium is effective either by substituting for and replacing insulin or by enhancing the responsiveness of tissues to low levels of endogenous insulin in the diabetic state. Vanadate treatment has been shown to stimulate hexose uptake into liver and muscle tissues of nondiabetic and STZ-D rats (11). Vanadate administration restored the glycogen and fructose-2,6-diphosphate content of liver in diabetic animals without having any effect in nondiabetic animals (12). Bollen et al. (40) observed normalization of glucose-induced activation of glycogen synthase in liver cells from vanadate-treated diabetic rats. A marked increase in the activities of both glycogen-synthesizing and glycogenolytic enzymes in the liver of diabetic rats after vanadate therapy has been reported (39), suggesting that vanadate restores glycogen turnover to near-normal levels. Muscle glycogen synthesis during the insulin clamp, which was reduced by ~80% in partially pancreatectomized diabetic rats, was restored to normal by vanadate (38). Strout et al. (41) recently showed that vanadate induces expression of the insulin-sensitive glucose transporter in the skeletal muscle of both diabetic and nondiabetic rats.

Considerable evidence suggests that insulin-receptor kinase activity plays an essential role in at least some of the intracellular actions of insulin (28,42–44). Accordingly, it has been proposed that vanadium may exert insulin-mimetic effects by maintaining insulin-receptor and other endogenous proteins in an appropriate state of phosphorylation by activating receptor kinase (7,45) and/or inhibiting phosphotyrosine phosphatase (36). Although, vanadate was shown to activate the intrinsic tyrosine kinase of highly purified insulin receptor in vitro (7), little or no increase in tyrosine phosphorylation of either insulin receptor or endogenous substrates was observed in intact cells treated with vanadate alone (1,46,47). Phosphotyrosine phosphatase activity in both cytosolic and particulate fractions was found to be elevated in the liver of STZ-D rats; treatment with either insulin or vanadate significantly lowered phosphatase activity in particulate fraction with no effect on the cytosolic phospha-

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tase activity (48). In vivo administration of vanadate to mice stimulated the incorporation of glucose into glycogen in mouse diaphragm, with no detectable activation of insulin-receptor kinase activity (37). The stimulatory effect of vanadate on hexose uptake was intact in adipocytes that had been treated with trypsin or insulin and Tris to decrease insulin-receptor concentration by 95 and 60%, respectively (49). Mooney et al. (50) observed that, although vanadate and insulin exerted comparable effects on isoproterenol-mediated lipolysis in rat adipocytes, their effects on tyrosine phosphorylation of insulin-receptor and endogenous proteins were dissimilar. Moreover, the corrective effect of vanadate on liver glucose metabolism in a rat model of non-insulin-dependent diabetes was not accompanied by a corresponding alteration in the activity of hepatic insulin-receptor kinase (51). Collectively, these findings suggest that vanadate elicits insulinlike effects by acting at a level distal to the insulin receptor.

In this study, insulin receptors were isolated from the tissues of rats that underwent submaximal hyperinsulinemic clamp study under conditions designed to preserve their phosphorylation state, and kinase activity was measured without further activating the receptor. These conditions ensured that the tyrosine kinase activity assayed in vitro reflected the in vivo activation state. Our data show that vanadyl treatment of diabetic rats did not alter the phosphorylation state of liver- and muscle-derived insulin receptors, although glucose uptake and utilization were markedly enhanced under the same conditions. In conclusion, our findings, along with those of other investigators, support a post–insulin-receptor mechanism of vanadium action.

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