Insulinlike Effects of Vanadate on Hepatic Glycogen Metabolism in Nondiabetic and Streptozocin-Induced Diabetic Rats

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The effect of oral administration of sodium orthovanadate for 5 wk on hepatic glycogen metabolism was studied in control and streptozocin-induced diabetic rats. Diabetes caused hyperglycemia (5-fold increase), hypoinsulinemia (85% decrease), and hyperglucagonemia (4-fold increase). There were also marked decreases in liver glycogen and activities of glycogen-metabolizing enzymes in liver. Although vanadate administration in control animals showed no significant effect on the various parameters measured except for a 70% decrease in plasma insulin, this treatment in diabetic rats restored these parameters to near control values. In diabetic rats, glycogen synthase *a* and the activity ratio (activity of glycogen synthase *a* divided by activity of total glycogen synthase) decreased to 30% of control levels and were restored to ~70–80% of control values after vanadate administration. A similar pattern was observed for the activity of synthase phosphatase. The activities of glycogenolytic enzymes, i.e., phosphorylase (activity of phosphorylase *a* and activity of total phosphorylase), phosphorylase kinase, and protein kinase (in presence or absence of cAMP), were significantly decreased by 40–70% in diabetic rats. These enzyme activities were recovered to 70–100% of control values after vanadate treatment. Phosphorylase phosphatase was not altered by diabetes, but the vanadate treatment of both groups, i.e., control and diabetic rats, showed a 25% increase in its activity ($P < 0.01$). In conclusion, these results show insulinlike in vivo action of vanadate on various parameters related to hepatic glycogen metabolism. *Diabetes* 39:821–27, 1990

Vanadate (a salt of vanadic acid, which is a derivative of vanadium) is an essential trace element of unknown function in cellular regulation and an endogenous constituent of most mammalian tissues (for review, see refs. 1 and 2). Vanadate has been shown to have insulinlike effects on metabolism of glucose by both in vivo (3–7) and in vitro (8–14) studies in various tissues. Oral administration of vanadate to streptozocin-induced diabetic (STZ-D) rats causes normalization of hyperglycemia (3,4) and tissue responsiveness to insulin (5). Vanadate treatments of diabetic rats produce insulinlike effects in liver by decreasing the activity of gluconeogenic enzyme glucose-6-phosphatase (6) and increasing the activities of glycolytic enzymes glucokinase and phosphofructokinase (7). In vitro, vanadate stimulates the transport and metabolism of glucose in adipocytes (8–11) and muscle (12,13). In hepatocytes, vanadate causes insulinlike (12,14) and anti-insulinlike (15) effects. Tolman et al. (12) observed an increased incorporation of glucose into glycogen in the presence of vanadate. Bosch et al. (15) reported the inactivation of glycogen synthase and activation of phosphorylase by vanadate, although in a later study, they showed stimulation of glycolysis (14).

Insulin plays a key role in the regulation of glycogen metabolism in liver. In an earlier study, we showed that STZ-D results in decreased activities of glycogen-metabolizing enzymes in liver, and insulin therapy restores these enzyme activities (16). No detailed information is available on the in vivo effects of vanadate on these enzymes. This study shows that vanadate treatment of STZ-D rats leads to insulinlike normalization of enzymes involved in liver glycogen metabolism.

RESEARCH DESIGN AND METHODS

Rabbit skeletal muscle phosphorylase *b* was prepared according to the method of Fischer and Krebs (17). Rabbit skeletal muscle phosphorylase kinase was prepared by the method of Hayakawa et al. (18). [^{32}P]phosphorylase *a* was prepared from phosphorylase *b* with [γ-^{32}P]ATP, Mg^{2+}, and phosphorylase kinase as described by Krebs et al. (19).

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[γ-^{32}P]ATP, [^{14}C]glucose-1-phosphate, UDP-[^{14}C]glucose, and Formula-963 for liquid-scintillation counting were obtained from New England Nuclear (Lachine, Canada). Sodium orthovanadate, UDP-glucose, glucose-1-phosphate, type III rabbit liver glycogen, AMP, 2-(*N*-morpholino)ethanesulfonic acid, type II histone, cAMP, rabbit skeletal muscle glycogen synthase , and STZ were products of Sigma (St. Louis, MO). Trasylol was obtained from Miles (Toronto). All other chemicals used were of analytical grade.

Six-month-old Sprague-Dawley female rats (300–350 g) were used in this study. They were fed a pelleted rodent chow (77% carbohydrate, 16% protein, 7% fat) ad libitum and kept under a constant 12-h light-dark cycle. The rats were made diabetic by a single injection of 60 mg/kg body wt i.p. STZ dissolved in 100 mM citrate (pH 4.5) containing 150 mM NaCl after 24 h of fasting. Control rats were injected with citrate buffer alone. The diabetic state was monitored by testing urinary glucose daily with Keto-Diastix strips (Miles, Toronto) and by determination of blood glucose levels once after 1 wk. The rats were divided into four groups of six each: untreated control, vanadate-treated control, untreated STZ-D, and vanadate-treated STZ-D rats. The untreated rats drank a 0.5-g NaCl/100 ml solution, whereas the vanadate-treated rats drank a 0.5-g NaCl/100 ml solution containing 0.5 mg/ml sodium orthovanadate freshly prepared every 3rd day. NaCl was given because it is known to reduce the toxicity of vanadate (3). Because the rats did not like the taste of vanadate, treatment was commenced at a concentration of 0.1 mg/ml and was increased gradually to 0.5 mg/ml over 1 wk. The treatment was continued at this dose for a further 4 wk. At the end of the treatment period, the rats were killed between 0900 and 1000 in fed condition. Blood was collected by orbital sinus bleeding in heparin and Trasylol. The blood samples were chilled on ice and centrifuged at 3000 × *g* for 10 min, and the plasma was stored at −80°C until used. The livers were frozen in liquid N_2 and later pulverized and stored at −80°C.

For all enzyme assays except phosphatases and phosphorylase kinase, rat livers were homogenized in 10 vol of 20-mM Tris-HCl (pH 7.4) containing 0.25 M sucrose, 0.05 mM dithiothreitol, and 50 mM NaF. In the case of phosphorylase phosphatase, the homogenization was in 10 vol of the same buffer without NaF. For the phosphorylase kinase assay, the homogenization buffer was the same but supplemented with a heat-stable inhibitor of protein kinase. For the glycogen-synthase-D phosphatase assay, liver samples were homogenized in 30 vol of 50-mM Tris-HCl (pH 7.8) containing 10 mM EDTA and 50 mM 2-mercaptoethanol. The homogenates were centrifuged at 10,000 × *g* for 20 min at 4°C, and the supernatants were used for the assay. For the assay of protein kinase, supernatants were passed through Sephadex G-25. All enzyme assays were carried out at 30°C.

Phosphorylase activity was measured in the direction of glycogen synthesis with [^{14}C]glucose-1-phosphate described by Tan and Nuttall (20). Glycogen synthase activity was determined by the method of Thomas et al. (21) by measuring the incorporation of [^{14}C]glucose from UDP-[^{14}C]glucose into glycogen. In the assay of these two enzymes, radioactive glycogen was precipitated on Whatman 31 ET paper, washed in 66% vol/vol ethanol, and counted for radioactivity. Protein kinase activity was assayed by following the incorporation of $^{32}P_i$ from [γ-^{32}P]ATP into type II-A histone in the presence and absence of cAMP as described by Reimann et al. (22). The radioactive histone was absorbed on phosphocellulose paper, washed in 0.5% phosphoric acid, and counted for radioactivity. The data on cAMP-dependent protein kinase were validated with the use of a heat-stable inhibitor of protein kinase. Phosphorylase kinase activity was determined by the amount of phosphorylase *a* formed from phosphorylase *b* essentially according to the methods described by Brostrom et al. (23) and Doorneweerd et al. (24). The activity of this phosphorylase *a* was measured in the direction of glycogen synthesis. Glycogen synthase phosphatase activity was determined with exogenously added rabbit skeletal muscle glycogen synthase *b* as the substrate by the method of Okubo et al. (25). The glycogen synthase *a* released is assayed by the method described above (21). Phosphorylase phosphatase activity was determined with ^{32}P-labeled phosphorylase *a* as the substrate with the preparation previously described (26). The released radioactive phosphate is measured in the supernatant after precipitating the enzyme with trichloroacetic acid.

Plasma glucose and alanine aminotransferase levels were measured with diagnostic kits from Sigma. Liver glycogen was determined by the method of Lo et al. (27). Plasma insulin was measured via the double-antibody method with a kit from Cambridge (Billerica, MA) (28). Plasma glucagon was determined by the method of Heding (29) with antiporcine glucagon rabbit serum K 5563 (Novo, Toronto). For vanadium estimation, liver and plasma samples were processed by the method of Stroop et al. (30), and the element was analyzed at the Saskatchewan Research Council (Saskatoon, Canada) by inductively coupled plasma mass spectrometry. Protein was estimated by the method of Lowry et al. (31) by use of bovine serum albumin as the standard. Statistical analysis was performed by Student's *t* test.

RESULTS

The food, fluid, and vanadate intake and body weight change in different groups of rats are summarized in Table 1. The body weight gain was lower in diabetic than in control rats during the experimental period. Vanadate treatment of control rats showed a significant decrease in body weight gain compared with untreated control rats ($P < 0.01$). In diabetic rats, vanadate treatment did not cause any gain in body weight, and in fact, there was a significant weight loss. Food intake was considerably less (30–45%) in vanadate-treated control and diabetic rats compared with their respective untreated groups. Diabetic rats consumed more food than control rats. Fluid intake in untreated diabetic rats was sevenfold higher than in untreated control rats. Vanadate treatment caused a decrease of 46 and 87% of fluid intake in control and diabetic rats, respectively. Vanadate consumption was 44 ± 3 and 85 ± 10 mg · kg^{-1} · day^{-1} in control and vanadate-treated diabetic rats, respectively, the difference being due to different volumes of fluid intake by the two groups of rats.

Vanadium levels in plasma and liver and the effect of vanadate treatment on plasma alanine aminotransferase, a marker of liver function, are shown in Table 2. Rats receiving vanadate treatment in control and diabetic groups had

TABLE 1
Body weight change and food, fluid, and vanadate intake in rats

	Body weight gain (g)	Food intake (g/day)	Fluid intake (ml/day)	Vanadate intake (mg · kg^{-1} · day^{-1})
Control				
Untreated	43 ± 9	24 ± 1	58 ± 1	
Vanadate treated	7 ± 1*	17 ± 1†	31 ± 2†	44 ± 3
Streptozocin-induced diabetic				
Untreated	24 ± 9	55 ± 2‡	407 ± 21‡	
Vanadate treated	−41 ± 17§	29 ± 2†	51 ± 6†	85 ± 10

Values are means ± SE. Comparisons were by Student's *t* test.
*$P < 0.01$, †$P < 0.001$, §$P < 0.05$, vs. untreated.
‡$P < 0.001$ vs. control.

plasma vanadium levels of 17.5 ± 5.3 and 22.0 ± 5.3 μM, respectively. The liver vanadium concentration was 15.9 ± 2.4 μmol/kg in treated control rats and 20.2 ± 4.1 μmol/kg in vanadate-treated diabetic rats. Diabetic rats had higher vanadium levels because of increased vanadate intake. Untreated rats showed very low levels of vanadium in plasma (<2.0 μM) and liver (<2.0 μmol/kg). Vanadate intake did not cause any liver damage in control and diabetic rats, as indicated by the normal levels of plasma alanine aminotransferase.

Plasma levels of glucose, insulin, and glucagon and hepatic concentrations of glycogen and soluble proteins in various groups of rats are summarized in Table 3. In control rats, vanadate treatment did not produce any change in plasma glucose concentration. STZ-D resulted in a fivefold increase in plasma glucose levels, and vanadate treatment of these rats caused a significant decrease (57%) in glucose levels. Vanadate-treated control rats had a low plasma insulin level of 97.0 ± 9.8 pM, compared to 283.3 ± 37.3 pM in untreated control rats. Diabetes caused a substantial ($P <$ 0.001) fall in plasma insulin levels, which were slightly increased after the vanadate treatment. There was a small but insignificant increase in plasma glucagon levels in vanadate-treated control compared with untreated rats. Diabetes resulted in a fourfold increase in plasma glucagon levels, which decreased by 66% after vanadate treatment. Insulin-glucagon molar ratio was 6.12 and 0.23 in untreated control and diabetic rats, respectively. Vanadate-treated control rats showed a ratio of 1.36, which was comparable to the value of 1.23 found in vanadate-treated diabetic rats. Liver glycogen content was decreased by 86% in diabetic rats compared with controls. Vanadate-treated diabetic rats showed a significant recovery ($P < 0.01$), and this level was comparable to vanadate-treated controls but was still lower than untreated controls. The soluble protein concentration of liver did not change significantly due to either diabetes or vanadate treatment.

Glycogen synthase activity measured in the absence (*a* form) and presence (total) of glucose-6-phosphate in various groups of rats is shown in Figure 1. STZ-D produced a significant fall ($P < 0.001$) in the active *a* form of the enzyme without any change in the total enzyme activity, resulting in a fall of the activity ratio (*a*/total) from 0.42 to 0.14 ($P <$ 0.001). After vanadate treatment, the active enzyme increased from 0.16 to 0.33 U · mg^{-1} protein · min^{-1} ($P < 0.01$) and the ratio from 0.14 to 0.34 ($P < 0.001$). However, the values were still below control levels. Vanadate treatment showed no effect on the glycogen synthase activity in control rats. Figure 2 shows the activities of phosphorylase (*a*, total, ratio) in the four groups of rats. In untreated diabetic rats, phosphorylase *a* activity decreased by 71% and total activity by 75%, with the result that the activity ratio remained the same. After vanadate treatment, the diabetic rats showed a 2.3-fold increase in *a* and total activities, but these values were still below control levels. As in the case of glycogen synthase, vanadate treatment did not result in any change in phosphorylase activities in control rats.

Table 4 summarizes the effect of STZ-D and vanadate treatment on the activities of kinases and phosphatases involved in hepatic glycogen metabolism. There was a significant decrease in the activity of protein kinase both in the presence ($P < 0.001$) and absence ($P < 0.05$) of cAMP in diabetic rats, and these levels were restored almost completely after vanadate treatment. These changes were re-

TABLE 2
Plasma and liver vanadium concentrations and plasma alanine aminotransferase activity in rats

	Plasma vanadium (μM)	Liver vanadium (μmol/kg)	Plasma alanine aminotransferase (U/L)
Control			
Untreated	<2.0	<2.0	31.3 ± 2.4
Vanadate treated	17.5 ± 5.3	15.9 ± 2.4	34.6 ± 3.8
Streptozocin-induced diabetic			
Untreated	<2.0	<2.0	41.7 ± 1.4
Vanadate treated	22.0 ± 5.3	20.2 ± 4.1	39.8 ± 3.3

Values are means ± SE.

TABLE 3
Effect of diabetes and vanadate treatment on plasma levels of glucose, insulin, and glucagon and hepatic concentrations of glycogen and soluble proteins

	Plasma glucose (mM)	Plasma insulin (pM)	Plasma glucagon (pM)	Liver glycogen (mg/g)	Liver-soluble proteins (mg/g)*
Control					
Untreated	6.0 ± 0.2	283.3 ± 37.3	46.3 ± 6.0	47.7 ± 5.7	94.1 ± 1.3
Vanadate treated	5.8 ± 0.5	97.0 ± 9.8†	71.2 ± 13.6	33.2 ± 4.2	93.9 ± 1.4
Streptozocin-induced diabetic					
Untreated	30.9 ± 0.8‡	40.8 ± 3.5‡	178.7 ± 30.7‡	6.7 ± 1.3‡	100.5 ± 3.6
Vanadate treated	13.2 ± 1.7†	74.5 ± 20.4	60.8 ± 7.0†	28.4 ± 5.0§	96.3 ± 1.4

Values are means ± SE. Comparisons were by Student's *t* test.
*10,000 × *g* supernatant.
†$P < 0.001$, §$P < 0.01$, vs. untreated.
‡$P < 0.001$ vs. control.

flected in a similar way on the activity of phosphorylase kinase. In diabetic rats, the enzyme activity was 57% less compared with controls, and there was a complete recovery after vanadate treatment. No significant effect of vanadate was observed on the activity of both kinases in control rats. The activity of synthase phosphatase in diabetic livers was significantly less ($P < 0.001$) compared with control values. Vanadate treatment brought a significant recovery from 0.31 ± 0.06 to 0.72 ± 0.04 U · mg^{-1} protein · min^{-1}, which was still less than the control level. Control and diabetic livers showed similar activities of phosphorylase phosphatase. However, vanadate treatment resulted in a significant increase ($P < 0.01$) in the enzyme activity in both groups.

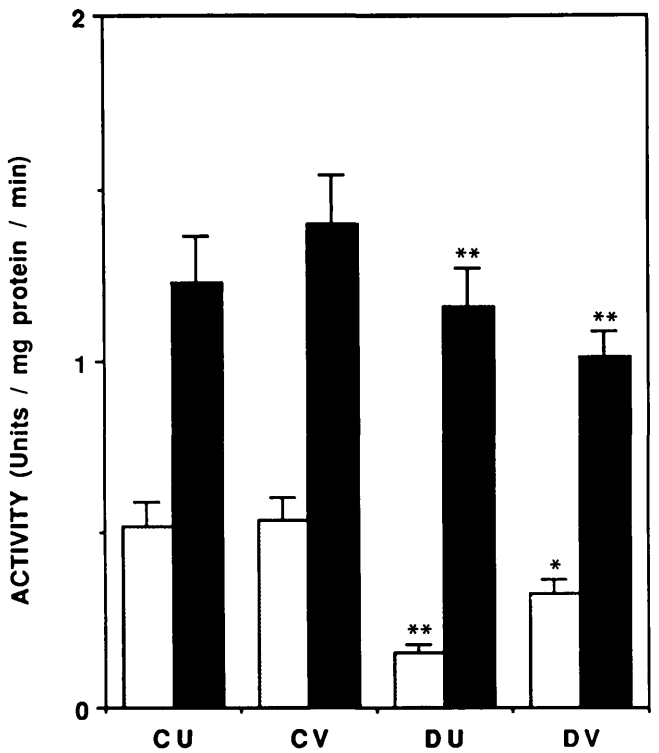

FIG. 1. Glycogen synthase *a* (*open bars*) and total glycogen synthase (*solid bars*) activities in untreated control (CU), vanadate-treated control (CV), untreated diabetic (DU), and treated diabetic (DV) rats. Activity ratios (amount of glycogen synthase *a* divided by amount of total glycogen synthase) were 0.42 ± 0.02 for CU, 0.399 ± 0.03 for CV, 0.14 ± 0.01 for DU, and 0.34 ± 0.03 for DV rats. One unit of glycogen synthase activity is amount of enzyme that incorporates 1 nmol [^{14}C]glucose from UDP-[^{14}C]glucose into glycogen. Values are means ± SE. Comparisons were by Student's *t* test. *$P < 0.01$ vs. DU. **$P < 0.001$ vs. CU.

DISCUSSION

The results of this study clearly indicate that vanadate has insulinlike effects on hepatic glycogen metabolism in vivo. Chronic treatment of diabetic rats with sodium orthovanadate caused significant improvement in hyperglycemia and recovery in the activities of glycogen-metabolizing enzymes in diabetic livers. These findings are in agreement with the insulinlike effects shown by earlier in vivo studies (5,7). However, they are in contrast to the in vitro findings of Bosch et al. (15), who showed an anti-insulinlike effect on glycogen synthase and phosphorylase in hepatocytes.

Vanadate decreased the body weight gain in control and diabetic rats due to decreased food intake. The decrease of body weight caused by vanadate in diabetic rats in our study is more than that reported by Heyliger et al. (3) but closer to the observation of Meyerovitch et al. (5). These differences are essentially due to differences in age and severity of diabetes in experimental animals used. Recently, Meyerovitch et al. (32) showed stimulation of glucose uptake in brain tissue by vanadate leading to decrease of food intake and body weight gain, and this is not viewed as a toxic effect. In a recent study, Brichard et al. (33) included a group of pair-fed control diabetic rats to show that vanadate-induced improvement in glucose homeostasis is not secondary to decreased food intake.

The hyperglycemia of diabetic rats was significantly ($P < 0.001$) improved by vanadate treatment, but plasma glucose concentrations were still higher than normoglycemic levels (Table 3). Some of the previous in vivo studies achieved normoglycemia by giving a higher dose of 0.8 mg/ml sodium orthovanadate (3,5,7). However, this higher dose has been shown to be toxic (5). In this study, therefore, a nontoxic suboptimal dose of 0.5 mg/ml sodium orthovanadate was given. Suppression of hyperglycemia is due to the combined effects of increased turnover of glycogen, stimulation of glycolysis, and inhibition of the gluconeogenic pathway. This study provides evidence for an improved hepatic glycogen metabolism by vanadate. Earlier studies showed increased activities of the glycolytic enzymes glucokinase and phosphofructokinase (7) and suppressed activities of the gluconeogenic enzyme glucose-6-phosphatase (6) in diabetic livers by vanadate. A stimulation of glycolysis in hepatocytes

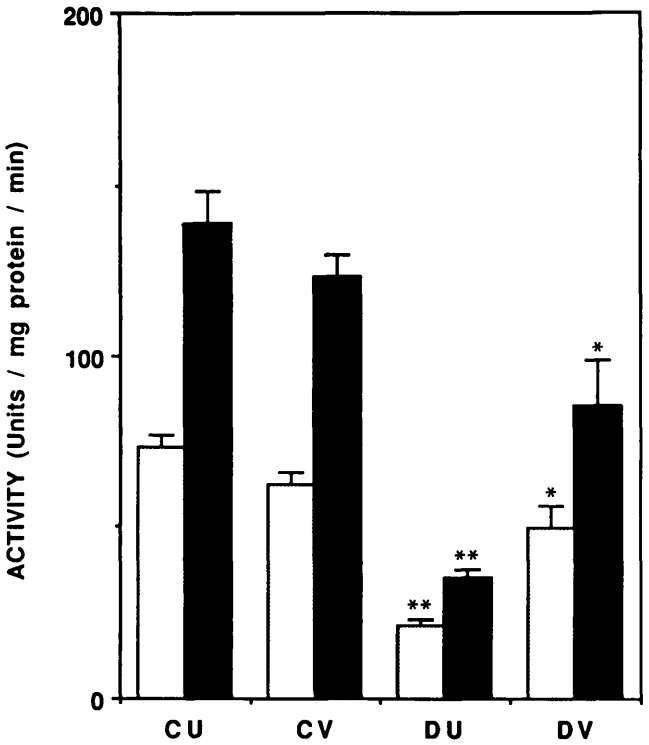

FIG. 2. Phosphorylase *a* (*open bars*) and total phosphorylase (*solid bars*) activities in untreated control (CU), vanadate-treated control (CV), untreated diabetic (DU), and treated diabetic (DV) rats. Activity ratios (amount of phosphorylase *a* divided by amount of total phosphorylase) were 0.54 ± 0.02 for CU, 0.52 ± 0.03 for CV, 0.61 ± 0.03 for DU, and 0.59 ± 0.03 for DV rats. One unit of phosphorylase activity is amount of enzyme that incorporates 1 nmol [^{14}C]glucose from [^{14}C]glucose-1-phosphate into glycogen. Values are means ± SE. Comparisons were by Student's *t* test. **$P < 0.001$ vs. CU. *$P < 0.01$ vs. DU.

by vanadate has also been reported by an in vitro study (14). However, in muscle, vanadate selectively mimics some of the actions of insulin (34).

In control rats, vanadate administration lowered the plasma insulin level significantly ($P < 0.001$; Table 3). Similar findings were reported by Heyliger et al. (3). This could be due to decreased insulin secretion caused by increased tissue responsiveness to insulin (4). Adipocytes derived from vanadate-treated rats have been shown to have increased sensitivity to insulin (5). The effects of vanadate independent of insulin could also be responsible for the decrease of plasma insulin in vanadate-treated control rats. In diabetic rats, vanadate did not change the plasma insulin level. However, the glucagon level decreased significantly, resulting in the increase of insulin-glucagon molar ratio ($P < 0.001$; Table 3). This could further enhance the insulinlike effects of vanadate in diabetic rats. A comparison of the plasma levels of insulin and glucagon in the four groups of rats indicate the presence of a physiological mechanism by which the secretion of these hormones is adjusted according to glucose homeostasis. Although insulin and glucagon regulate the opposing pathways under acute conditions (i.e., glycogen synthesis and breakdown, respectively), the chronic deficiency of insulin seen in diabetes leads to impairment of both pathways. There is a decrease of glycogenolytic enzymes in diabetic livers despite hyperglucagonemia.

Liver glycogen content improved significantly ($P < 0.01$) in treated diabetic rats (Table 3). The active *a* form of glycogen synthase and the activity ratio (*a*/total) showed a near-twofold increase in diabetic rats without any change in total enzyme activity after the treatment. Similar increases in the activity of synthase phosphatase were also observed (Table 4). The fall of glycogen synthase *a* in STZ-D rats is reported to be due to decreased synthase phosphatase (16). Glycogen synthase *b* isolated from alloxan-induced diabetic rats was found to be a poorer substrate for synthase phosphatase (35), and a defect in the G component (associated with a glycogen particle) of synthase phosphatase has also been identified (36). Hence, the recovery of synthase phosphatase after vanadate treatment could be due to its action on either or both sites.

Diabetes was associated with the decrease of phosphorylase (*a* and total), cAMP-dependent protein kinase, and phosphorylase kinase activities. However, phosphorylase phosphatase activity did not change significantly. After treatment of diabetic rats with vanadate, phosphorylase (*a* and total) activities increased by more than twofold without any change in the activity ratio (*a*/total). The cAMP-dependent

TABLE 4
Effect of diabetes and vanadate treatment on protein kinase, phosphorylase kinase, synthase phosphatase, and phosphorylase phosphatase activities in liver

	Protein kinase		Phosphorylase kinase	Synthase phosphatase	Phosphorylase phosphatase
	No cAMP	Plus cAMP			
Control					
Untreated	26.3 ± 3.5	104.5 ± 11.7	289.4 ± 18.7	0.94 ± 0.04	1.17 ± 0.05
Vanadate treated	21.8 ± 2.5	86.7 ± 5.1	340.2 ± 24.5	0.82 ± 0.07	1.41 ± 0.05*
Streptozocin-induced diabetic					
Untreated	17.7 ± 1.6†	47.7 ± 3.5‡	124.3 ± 9.2‡	0.31 ± 0.06‡	1.15 ± 0.05
Vanadate treated	27.8 ± 3.2§	83.2 ± 7.8‖	329.3 ± 27.8‖	0.72 ± 0.04‖	1.42 ± 0.06*

One unit of protein kinase activity is defined as the amount of enzyme that incorporates 1 pmol of $^{32}P_i$ from [γ-^{32}P]ATP into histone per minute. One unit of phosphorylase kinase activity is that amount of enzyme that converts 1 mU of phosphorylase *b* into phosphorylase *a* per minute. One unit of glycogen synthase phosphatase activity is the number of units of glycogen synthase *a* (exogenous enzyme) generated per minute. One unit of phosphorylase phosphatase activity is the amount of enzyme that releases 1 pmol of $^{32}P_i$ from ^{32}P-labeled phosphorylase *a* per minute. All enzyme activities are expressed per milligram of protein of liver homogenate used. Values are means ± SE. Comparisons were by Student's *t* test.
*$P < 0.01$, §$P < 0.05$, ‖$P < 0.001$, vs. untreated.
†$P < 0.05$, ‡$P < 0.001$, vs. control.

protein kinase activity increased by 74% and phosphorylase kinase by 165%. Vanadate treatment also caused an increase in phosphorylase phosphatase that could imply an antiglycogenolytic effect. However, the regulatory subunit of type I cAMP-dependent protein kinase was shown to inhibit dephosphorylation of phosphorylase *a* catalyzed by phosphatases (37). Hence, the changes in kinases might have a predominant effect on glycogenolysis. From an overall view of the changes in glycogen-metabolizing enzymes in diabetic and vanadate-treated rats, it can be hypothesized that there is a poor turnover of glycogen as a result of insulin insufficiency, and vanadate restores it back to near control levels, as shown by insulin treatment in a previous study (16).

Vanadate was shown to exert a glycogenolytic anti-insulinlike effect on glycogen synthase and phosphorylase in rat hepatocytes over 20 min by Bosch et al. (15). Gomez-Foix et al. (14) also reported stimulation of glycolysis during that time. Tolman et al. (12) reported increased glycogen accumulation in the presence of vanadate on prolonged incubation. Vanadate seems to increase glucose oxidation in the initial phase and facilitate glycogen synthesis later. Hence, the activation of phosphorylase and inactivation of glycogen synthase by vanadate could be only a transient effect. In vitro, vanadate stimulates adenylate cyclase activity (2) and inhibits glycolytic enzymes, which are also anti-insulinlike (38). The discrepancies between the in vivo effects and the results of in vitro studies could be due to the distinct effects of different forms of vanadium (vanadate and vanadyl). Vanadate enters the cell by the anion transport mechanism and reduces to vanadyl ions. Furthermore, in vivo concentrations of vanadium in plasma and liver are in micromolar concentration (Table 2), whereas most of the in vitro experiments have been carried out with millimolar concentration of this ion. Vanadate has been shown to accumulate inside the cell in addition to vanadyl ions when higher concentrations are used (39).

The most extensively studied effect of vanadate is its potent inhibitory action on Na^+-K^+-ATPase activity in purified enzyme preparations (40) and intact cells (41). However, Dubyak and Kleinzeller (9) showed that the insulinlike action of vanadate in adipocytes was not due to its action on Na^+-K^+-ATPase, because the adipocyte converts the accumulated vanadate to the vanadyl form, which is incapable of inhibiting this enzyme. Several reports showed that vanadate can phosphorylate and activate the tyrosine kinase activity of the insulin receptor in adipocytes (10,11,42), placenta, and liver (43). Spontaneous esterification of tyrosine residues with vanadate has been reported, which could be responsible for the activation of tyrosine kinase activity of the insulin receptor (44). Vanadate has also been shown to inhibit phosphotyrosyl protein phosphatase activity (45,46). This effect would decrease the dephosphorylation of phosphorylated β-subunits of the insulin receptor and other endogenous substrates (45). In contrast to these findings, Mooney et al. (47) observed that the insulinlike effect of vanadate on lipolysis in rat adipocytes is not accompanied by a similar action on the phosphorylation of the insulin receptor and other intracellular proteins. Green (48) made an interesting observation that the insulinlike effect of vanadate could be observed in adipocytes that had been treated to decrease the number of insulin receptors, suggesting a postreceptor action. The diverse actions of various forms of vanadate under different experimental conditions make it difficult to propose a unified mode of its action. Further studies are therefore needed to precisely understand its insulin-mimetic actions and dissociate them from other nonspecific actions on other phosphate-transfer reactions.

Vanadate has been essentially used as a tool in understanding the mode of the action of insulin. However, the possibility that it could be used in the treatment of diabetes mellitus has been suggested (5). This study further supports such a possibility. In earlier studies, a high dose of vanadate (0.8 mg/ml in drinking water) was used, causing toxic effects to liver function (5,7). We reduced the dose to 0.5 mg/ml, which did not cause any damage to liver, as indicated by the normal plasma level of alanine aminotransferase. However, the dose was suboptimal and did not achieve complete normalization of some of the parameters studied. Therefore, further studies are needed to investigate whether nontoxic lower levels of vanadate can be given in combination with insulin or other oral hypoglycemic drugs for normalization of glucose homeostasis in diabetic animals. In addition, future studies need to be performed at more than one time point in fed and fasted rats on specific aspects of glycogen metabolism.

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