Concentration-Dependent Glucose-Lowering Effects of Oral Vanadyl Are Maintained Following Treatment Withdrawal in Streptozotocin-Diabetic Rats

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We have recently reported that treatment with vanadyl sulfate 0.75 mg/mL in drinking water eliminates hyperglycemia in a subset of streptozotocin (STZ)-diabetic rats, with some rats remaining unresponsive to such treatment. In the present study, we demonstrate that unresponsive diabetic animals become normoglycemic when given higher concentrations of vanadyl. Since the subset of rats that require higher concentrations ([HC] 1.25 to 1.50 mg/mL) were found to be more severely diabetic before treatment than those that responded to lower concentrations ([LC] 0.75 to 1.00 mg/mL), the relative amount of residual circulating insulin (LC 36.0 ± 2.2 *v* HC 25.6 ± 3.3 μU/mL) appeared to be a key element in achievement of a normoglycemic effect to a given dose of vanadyl. Similarly, STZ-diabetic animals that responded to euglycemia with a more potent organic vanadyl compound (naglivan) had higher pretreatment plasma insulin levels than unresponsive animals (DT-R) (35.5 ± 1.9 *v* 24.2 ± 3.6 μU/mL). Vanadyl treatment over 10 weeks resulted in a period of normalized glucose levels and glucose tolerance after treatment was stopped. At 20 weeks after withdrawal from treatment with vanadyl sulfate, 13 of 19 animals remained euglycemic, whereas four of seven naglivan-treated animals also maintained normal glucose levels after a 30-week withdrawal period. At 3 weeks after withdrawal, maintenance of normal glucose homeostasis appeared to be independent of altered insulin levels, whereas at 20 weeks an improved insulin secretion, albeit 50% that of age-matched controls both in the fed state and in response to a glucose dose, was sufficient to return plasma glucose levels to the normal range. In conclusion, the results suggest that in diabetic animals vanadyl and endogenous insulin work in an interdependent and complementary manner, and that maintenance of normal glucose homeostasis after withdrawal appears to be due to a continued enhancement of insulin sensitivity in the short term, whereas it may depend more critically on an improved pancreatic function in the long term.

AFTER THE INITIAL DISCOVERY by Heyliger et al[1] of the glucose-lowering and cardioprotective effects of sodium orthovanadate orally administered in streptozotocin (STZ)-diabetic rats, there has been a growing interest in the potential use of vanadium in diabetic therapy. Vanadium has been demonstrated to activate glucose transport in a variety of tissue types: adipocytes,[2,3] skeletal muscle,[4] and in vivo in brain,[5] liver,[6] and muscle.[6] In vitro, it has been reported to stimulate various enzymes in the glycolytic pathway, including glucose-6-phosphatase,[7] 6-phosphofructo-2-kinase,[8] and phosphoenolpyruvate carboxykinase.[9]

Oral administration of vanadyl (+IV)[10] or its organic derivatives[11,12] has been reported to produce marked glucose-lowering effects in STZ-diabetic rats. However, it was noted that treatment with vanadyl sulfate 0.75 mg/mL in drinking water did not produce euglycemia entirely in all the diabetic animals.[13] We postulated that the relative efficacy of vanadyl treatment may be dependent in part on a critical level of circulating insulin and preservation of a limited number of functional β cells. This is because rats made diabetic with STZ 55 mg/kg, although hypoinsulinemic, still have measurable amounts of circulating insulin in the plasma.[14] In addition, since the hypoinsulinemic action of STZ is markedly influenced by several factors including age, sex, metabolic status, and route of injection, this could result in diabetic rats with various degrees of hypoinsulinemia and hyperglycemia.[15] Hence, the apparent lack of a glucose-lowering response to vanadyl in some diabetic rats may have been due to a more severe diabetic state and lower levels of circulating insulin, which could be enhanced or complemented by increasing vanadium intake. In support of this idea, treatment of spontaneously diabetic BB Wistar rats (which have no measurable levels of circulating insulin[16]) with vanadyl diminishes the requirement for insulin, but does not replace insulin therapy altogether.[17]

One distinctive result of vanadyl treatment in STZ-diabetic rats is a sustained normal plasma glucose level and cardiac and adipose tissue function up to 13 weeks after treatment is withdrawn.[18] However, vanadyl treatment was started 3 days after STZ injection, a point at which it could be argued that its β-cell–cytotoxic effects may not have yet produced a significantly decreased islet insulin content.[19] This would allow vanadyl treatment to exert early protective effects on insulin-producing cells, which could subsequently release enough insulin to bring about a quasi-permanent euglycemic state after treatment is stopped. Indeed, in animals in which postwithdrawal euglycemia was maintained, the area occupied by insulin-staining β cells, although diminished relative to control values, was increased eightfold as compared with that in nontreated diabetic rats,[19] which suggests that vanadyl treatment somehow activated β-cell regeneration, or through its glucose-lowering effects prevented the continued destruction of β cells by hyperglycemia. Hence, the purpose of the present study was threefold: (1) to examine the existence of an in vivo dose-dependent effect of vanadyl, (2) to confirm if previous findings of maintained euglycemia after termination of vanadyl treatment apply to diabetic rats in which the start of treatment was delayed up to 17 days after STZ

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injection, and (3) to extend the observation of postwithdrawal euglycemia to a longer period.

MATERIALS AND METHODS

Treatment and Maintenance of Animals

Male Wistar rats (180 to 220 g) were injected intravenously with STZ 55 mg/kg in the tail vein. After 3 days, diabetic rats (DT, plasma glucose >13 mmol/L) were grouped according to when vanadyl treatment was initiated: at 3 ([DT3] n = 8), 10 ([DT10] n = 6), and 17 ([DT17] n = 5) days after diabetes was induced. The initial concentration of vanadyl sulfate trihydrate ($VOSO_4 \cdot 3H_2O$, Fisher Scientific, Fairlawn, NJ) supplied in drinking water was 0.75 mg/mL and was increased by 0.25-mg/mL increments on a weekly basis in those animals that did not respond to the point of euglycemia (plasma glucose < 9 mmol/L). The highest concentration attained was 1.50 mg/mL. A control group ([CT] n = 3) was administered vanadyl sulfate 1.0 mg/mL. Food intake, fluid intake, body weight, and plasma glucose level were monitored. At the end of a 10-week treatment period, vanadyl was withdrawn from all groups and plasma was collected for measurement of cholesterol, triglyceride, and vanadium levels. After treatment withdrawal, animals were monitored at regular intervals for 20 weeks. Plasma samples for measurement of insulin and glucose levels were periodically collected at 10 AM.

A separate group of diabetic animals were treated with naglivan [bis(cysteine, amide *N*-octyl)oxovanadium IV], an organic vanadyl compound that has been found to be more orally potent than vanadyl sulfate.[11] The compound was formulated as a 10-mg/mL suspension in 3% acacia. Rats were initially administered naglivan 50 mg/kg/d by oral gavage for 5 weeks, at which time the dose was increased to 100 mg/kg/d in those animals that remained hyperglycemic. After 3 subsequent weeks, the naglivan dose was again increased to 150 mg/kg/d in noneuglycemic animals. Because of the limited supply of naglivan, we did not further increase the dose in those rats that remained hyperglycemic. At 10 weeks, naglivan treatment was withdrawn, and animals were monitored up to 30 weeks after the withdrawal date.

Oral Glucose Tolerance Test

Before and 3 and 20 weeks after withdrawal from vanadyl treatment, oral glucose tolerance tests (OGTTs) were performed on overnight-fasted rats. A 1-g/kg oral glucose dose was provided via gavage. Blood was collected from the tail vein at 0, 10, 20, 30, 60, and 120 minutes for determination of plasma glucose and insulin levels. For naglivan-treated rats, the OGTT was performed at 30 weeks after withdrawal from treatment.

Analytical Methods

Plasma vanadium levels were measured as previously described via atomic absorption spectrophotometry.[20] Plasma glucose, cholesterol, and triglyceride levels were measured with kits (Boehringer Mannheim Canada, Laval, Quebec). Plasma urea nitrogen and aspartate aminotransferase levels were measured using kits from Sigma (St Louis, MO). Plasma insulin level was measured using rat insulin standards (NOVO, Copenhagen, Denmark). The radioimmunoassay method allows for measurement of small sample volumes of 25 μL, with an interassay and intraassay coefficient of variation of less than 15% and a sensitivity to 10 μU/mL.

Statistical Analysis

Data are the mean ± SEM unless otherwise specified. Statistical significance was evaluated by one-way ANOVA followed by the Neuman-Keuls test or Student's paired *t* test, where appropriate. *P* less than .05 was considered significant.

Table 1. Number of Euglycemic Animals at the End of 10-Week Treatment Period According to Vanadyl Concentration in Drinking Water

	Vanadyl Concentration (mg/mL)			
	0.75	1.00	1.25	1.50
DT3 (n = 8)	2	5	1	
DT10 (n = 6)	1	1	1	3
DT17 (n = 5)	1	3	1	
Total (N = 19)	4	9	3	3

RESULTS

Various Parameters of Euglycemic Animals Treated With Vanadyl Sulfate

After a 10-week treatment period, vanadyl sulfate at concentrations of 0.75 to 1.50 mg/mL in drinking water resulted in normal plasma glucose levels (<9 mmol/L; Table 1) in all diabetic animals. Since there was no difference in several parameters according to treatment time, data were pooled and analyzed according to the vanadyl concentration at which euglycemia was achieved. This resulted in four concentration groups (in milligrams per milliliter): [0.75], [1.00], [1.25], and [1.50]. A delay in the start of treatment to 17 days after STZ injection did not significantly increase the requirement for higher concentrations ([1.25] and [1.50]) as compared with lower concentrations ([0.75] and [1.00]) to reach euglycemia. The initial vanadyl dose (loading dose) was equivalent among concentration groups, approximately 140 to 150 mg/kg/d, or 0.65 to 0.70 mmol/kg/d (Fig 1). Over several weeks, there was a reduction in the calculated dose of vanadyl as a result of increasing body weight. The dose curve for CT (at 1.0 mg/mL) was similar to that for DT at the same concentration ([1.00], data not shown). At week 10, there was a

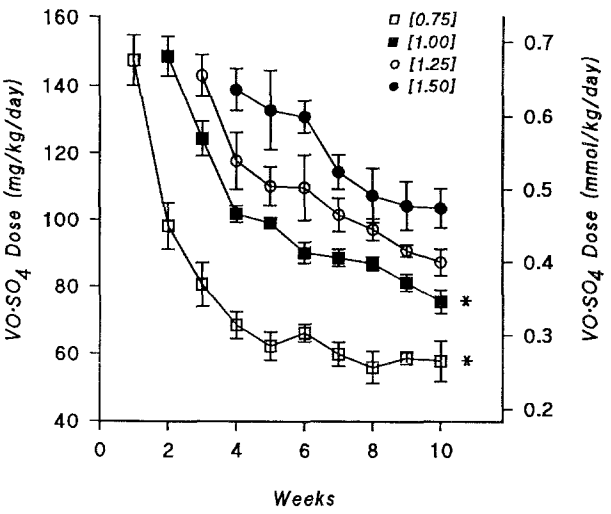

Fig 1. Calculated daily dose of vanadyl in DT normalized on various concentrations (mg/mL) of vanadyl sulfate in drinking water. *P < .05 v [1.50].

Table 2. Characteristics of Animals at the End of 10-Week Treatment Period

	Vanadyl Concentration (mg/mL)				
	Control	Diabetic			
	1.00 (n = 3)	0.75 (n = 4)	1.00 (n = 9)	1.25 (n = 3)	1.50 (n = 3)
Body weight gain (g)	151 ± 12	173 ± 22	168 ± 16	136 ± 18	149 ± 22
Food intake (g/rat/d)	26 ± 1	25 ± 1	24 ± 1	24 ± 1	22 ± 2*
Fluid intake (mL/rat/d)	31 ± 2	31 ± 3	32 ± 2	28 ± 3	24 ± 2*
Glucose (mmol/L)	5.7 ± 0.1	7.4 ± 0.2*	8.2 ± 0.3*	8.6 ± 0.1*	7.5 ± 0.4*
Insulin (μU/mL)	28 ± 10	27 ± 3	24 ± 2	27 ± 4	21 ± 4
Cholesterol (mmol/L)	1.5 ± 0.1	1.4 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.8 ± 0.1*
Triglycerides (mmol/L)	1.3 ± 0.2	1.4 ± 0.4	1.3 ± 0.2	1.4 ± 0.1	1.1 ± 0.1

*$P < .05$ *v* control.

concentration-dependent increase in the vanadyl dose ([0.75] < [1.00] < [1.25] ≈ [1.50]). There also appeared to be a concentration-dependent reduction in food and fluid intake, which was significantly lower in [1.50] as compared with CT (Table 2). We have previously reported that plasma cholesterol and triglycerides are elevated in untreated STZ-diabetic rats over a similar period.[13] In the present study, plasma cholesterol and triglycerides were not different from control values in most concentration groups, with the exception of [1.50], in which plasma cholesterol levels remained elevated. There was no correlation between vanadyl concentration and body weight gain or plasma vanadium levels, which were in the range of 0.26 to 1.05 μg/mL. However, there was a weak negative correlation between plasma insulin and vanadium levels after 10 weeks of treatment ($r = -.40$, $P = .06$, data not shown).

Plasma Glucose and Insulin Profile Before and After Treatment

A range in the severity of diabetes induced by STZ 55 mg/kg was reflected in a gradient of plasma insulin and glucose levels before vanadyl treatment, which was negatively correlated ($r = -.70$, $P < .001$). There appeared to be a normal distribution in the degree of diabetes among groups. However, when retrospectively examined according to concentration, the more severely diabetic animals (more pronounced hyperglycemia and hypoinsulinemia before treatment) appeared to require higher concentrations of vanadyl to achieve euglycemia. Thus, when diabetic animals were grouped according to the vanadyl concentration at which glucose levels were normalized, ie, low-concentration ([LC] 0.75 to 1.0 mg/mL) and high-concentration ([HC] 1.25 to 1.50 mg/mL) responders, it was found that HC had significantly higher plasma glucose (HC 19.6 ± 0.8 *v* LC 16.4 ± 0.5 mmol/L; Fig 2A) and correspondingly lower plasma insulin (HC 25.6 ± 3.3 *v* LC 36.0 ± 2.2 μU/mL; Fig 2B) relative to LC before vanadyl treatment. However, after the 10-week treatment period, there were no significant differences in either plasma glucose or insulin levels between LC and HC. Whereas long-term vanadyl treatment reduced plasma insulin in LC, there was no further reduction in plasma insulin in HC.

Changes in Plasma Glucose and Insulin After Withdrawal From Vanadyl Treatment

At 3 weeks after withdrawal from vanadyl, there were no differences in either mean plasma glucose or insulin levels in both LC and HC from the time before withdrawal. However, at 20 weeks after treatment withdrawal, mean plasma glucose in HC had increased to pretreatment values. Indeed, hyperglycemia was initially observed to occur after withdrawal in [1.50] as early as the first week, which was followed by [1.25] only at the fifth month. After withdrawal from treatment, there was a continuous in-

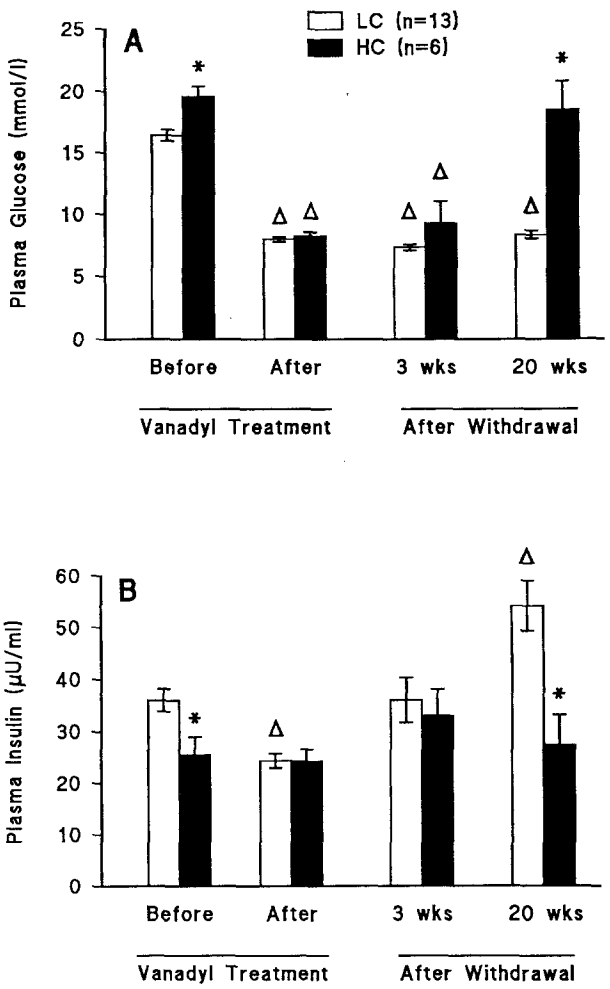

Fig 2. Plasma glucose (A) and insulin (B) levels before and after a 10-week period of vanadyl treatment and at 3 and 20 weeks after withdrawal from treatment in DT normalized with low concentrations (LC, [0.75] to [1.00]) and high concentrations (HC, [1.25] to [1.50]) of vanadyl sulfate. $^{\Delta}P < .05$ *v* before treatment; *$P < .05$ *v* LC.

crease in plasma insulin in CT, which reached 100 μU/mL by 20 weeks (data not shown). Alternately, in LC there was a more moderate increase in plasma insulin levels, reaching twice that of initial prewithdrawal levels (54.1 ± 4.9 *v* 24.3 ± 1.4 μU/mL) by 20 weeks. On the other hand, plasma insulin in HC at 5 months was approximately half the level in LC and was not significantly different from prewithdrawal level (27.5 ± 5.6 *v* 25.6 ± 3.4 μU/mL).

After withdrawal from vanadyl treatment, six of 19 diabetic animals reverted to the hyperglycemic state. Notably, this group included all the [1.50] and two of three [1.25]. Individual results after withdrawal from treatment in [1.50] are shown in Fig 3A to C. In these animals, it appeared that plasma insulin influenced coexisting glucose levels. For instance, in Fig 3A, wherein hyperglycemia recurred within the first week of withdrawal, there was no appreciable change in plasma insulin values from prewithdrawal levels. In Fig 3B, an 8-week period of normoglycemia was observed, during which time circulating insulin was stable for the first 5 weeks and subsequently increased twofold over 3 weeks. After 8 weeks, a reduction in insulin accompanied a rapid increase in glucose, whereas a further decline in insulin levels over 10 weeks reflected steady development of hyperglycemia. In Fig 3C, normal glucose was maintained for 8 weeks, whereas there was a marked threefold increase in insulin. However, concurrent with the return to hyperglycemia, plasma insulin decreased gradually over 10 weeks to prewithdrawal levels.

At 5 months after withdrawal, several plasma parameters were elevated in HC relative to control and were correlated with plasma glucose values. Plasma triglyceride levels were significantly greater in HC relative to LC and CT (HC 2.0 ± 0.4 *v* LC 1.3 ± 0.1 and CT 1.4 ± 0.3 mmol/L, $P < .05$). Similarly, plasma cholesterol levels were significantly greater in HC (HC 3.1 ± 0.4 *v* LC 2.3 ± 0.1 and CT 2.3 ± 0.2 mmol/L, $P < .05$). Plasma urea nitrogen levels were also greater in HC relative to the other groups (HC 9.2 ± 0.1 *v* LC 5.8 ± 0.2 and CT 6.7 ± 0.1 mmol/L, $P < .05$). However, plasma aspartate aminotransferase levels were not different among the various groups at 20 weeks after withdrawal from vanadyl.

Integrated Glucose and Insulin Response at 3 and 20 Weeks After Withdrawal From Vanadyl

To investigate sustained effects of vanadyl on glucose tolerance after withdrawal from treatment, OGTTs were conducted at 3 weeks (short-term, OGTT 1) and 20 weeks (long-term, OGTT 2) after vanadyl treatment was terminated. Also, an OGTT was performed at 10 weeks of treatment, before withdrawal (OGTT 0, data not shown). At OGTT 1, the total glucose responses in both LC and HC were significantly greater than in CT (Fig 4A). Although the glucose response of LC at OGTT 1 was greater than that seen at OGTT 0, there was no difference in HC (data not shown). After long-term withdrawal (OGTT 2), the glucose response in HC was significantly greater than in CT, whereas LC was not different from CT and not significantly different from OGTT 0. The total insulin response for LC and CT was not different between OGTT 1 and OGTT 0,

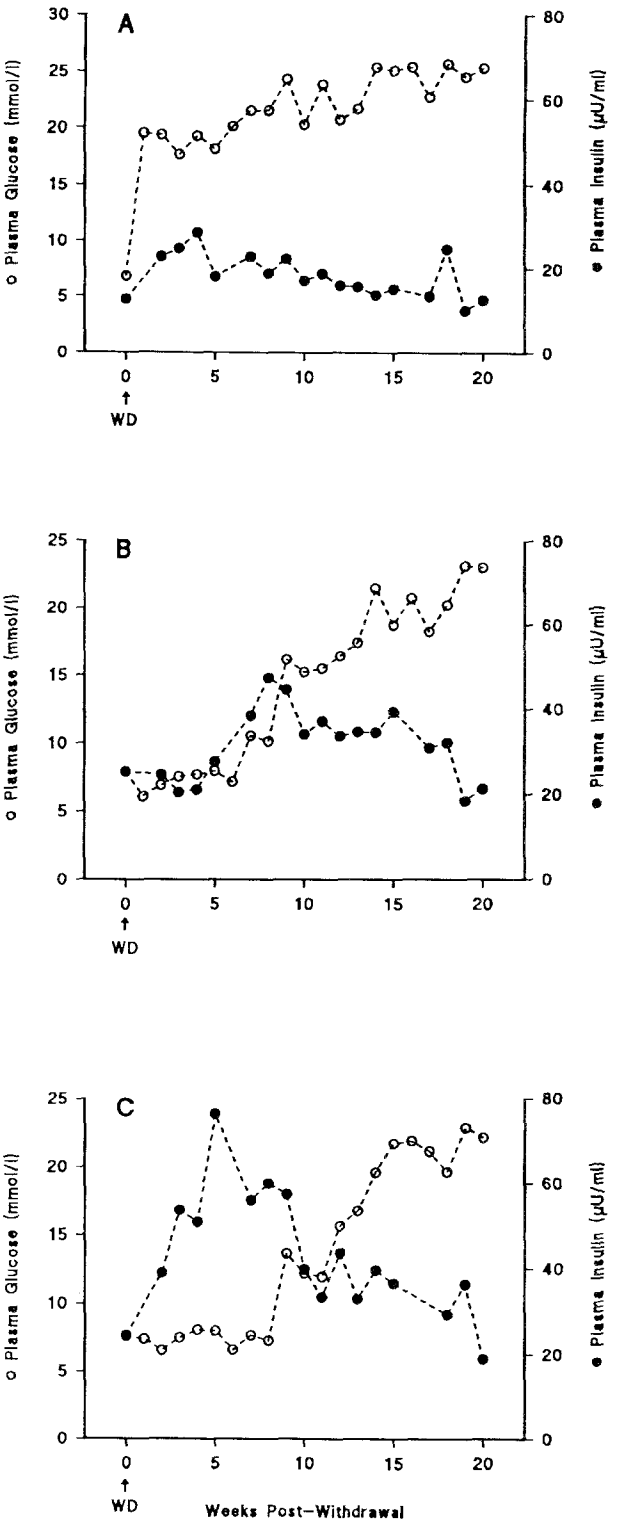

Fig 3. Plasma glucose and insulin levels over 20 weeks after withdrawal (WD) from vanadyl treatment in three DT (A through C) normalized at 1.50 mg/mL.

whereas there was a significant increase in both groups in OGTT 2 relative to OGTT 1 (Fig 4B). However, the insulin response in LC remained significantly lower than in CT at the 3- and 20-week withdrawal periods. On both tests, the

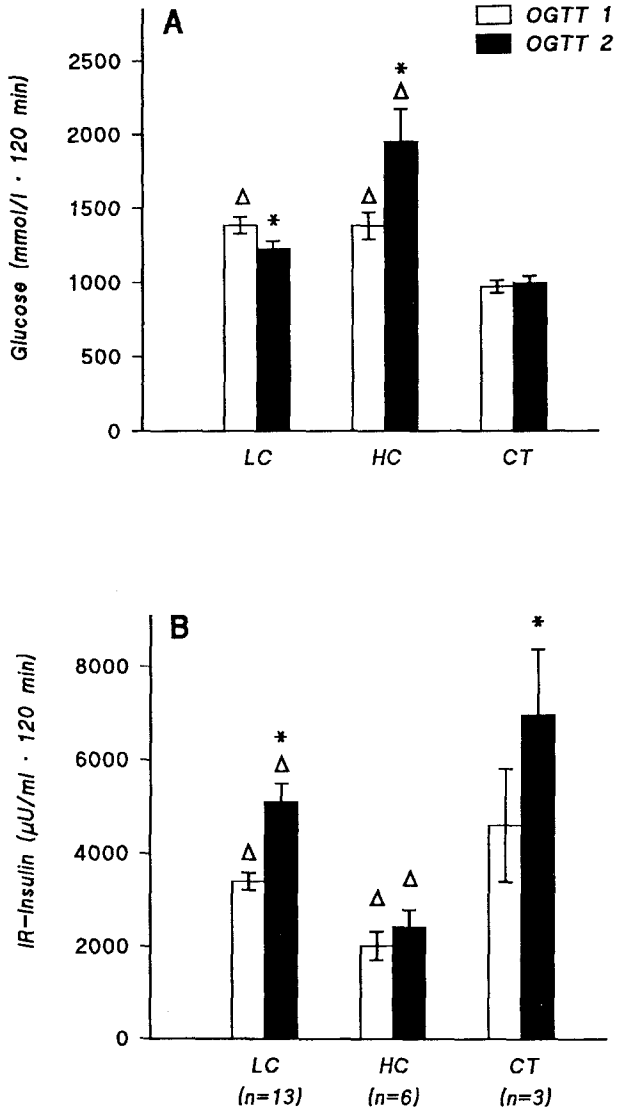

Fig 4. Integrated glucose (A) and insulin (B) responses from 3 weeks (OGTT 1) and 20 weeks (OGTT 2) after withdrawal from treatment of CT and DT normalized on low (LC) and high (HC) concentrations of vanadyl. *$P < .05$ v OGTT 1; $^{\Delta}P < .05$ v CT.

insulin response in HC did not differ from that at OGTT 0 and remained markedly lower than in CT and LC.

The degree of glucose tolerance after long-term withdrawal from vanadyl, as measured by total glucose response, was significantly correlated with fed glucose levels at 20 weeks ($r = .83, P < .0001$). Also, the insulin response to an oral glucose load at OGTT 2 correlated significantly with fed insulin levels at 20 weeks ($r = .69$, $P < .05$). To determine whether glucose tolerance was dependent on insulin response after short- and long-term withdrawal from vanadyl, we plotted the individual glucose response against insulin response during OGTTs. Whereas there was no significant correlation between glucose and insulin responses at the 3-week withdrawal period ($r = -.17$, $P = .448$; Fig 5A), there was a significant negative correlation ($r = -.74$, $P < .0001$; Fig 5B) between the integrated glucose and insulin response at 20 weeks after withdrawal from vanadyl.

Glucose-Lowering Effects of the Organic Vanadyl Compound Naglivan

Administration of naglivan 50 mg/kg/d (vanadium 0.06 mmol/kg/d) resulted in normal glucose levels in three diabetic animals (Fig 6A). After increasing the dose to 100 mg/kg/d in the four remaining noneuglycemic rats, only one developed euglycemia. When the naglivan dose was further increased to 150 mg/kg/d in the three that remained noneuglycemic, there was a slight decrease of plasma glucose but these rats remained hyperglycemic. Thus, animals were grouped according to response to treatment: euglycemic (responders [DT-R], n = 4) and hyperglycemic (nonresponders [DT-NR], n = 3). After naglivan (50 mg/kg/d) had been administered for 5 days, plasma insulin values were significantly higher in DT-R

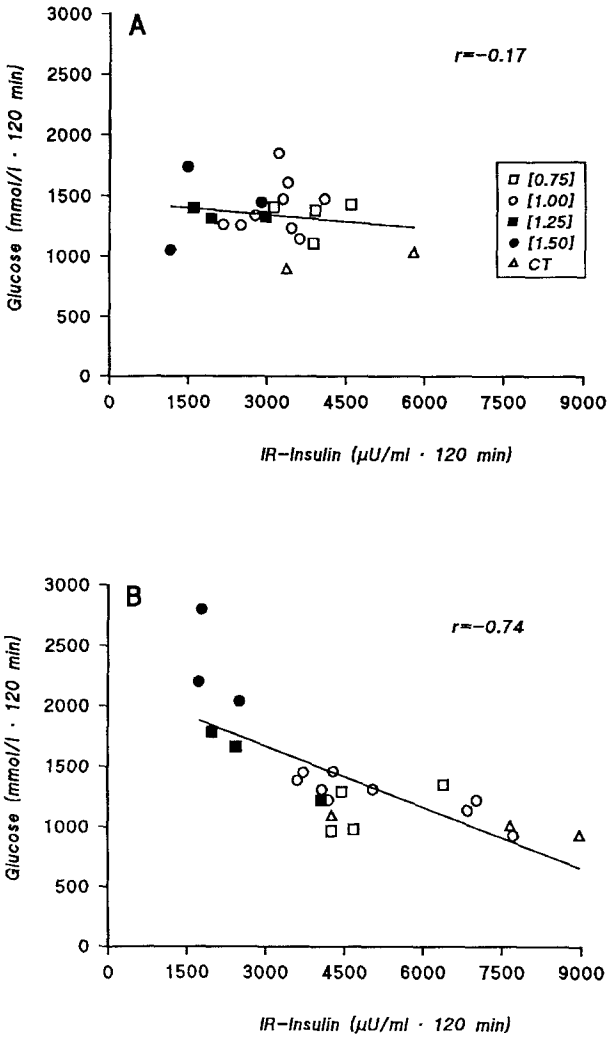

Fig 5. Correlation plots of integrated glucose response v total insulin response at (A) 3 ($r = -.17$, $P = .448$) and (B) 20 ($r = -.74$, $P < .0001$) weeks after withdrawal from treatment in CT and DT according to vanadyl concentration (mg/mL) at which plasma glucose was normalized.

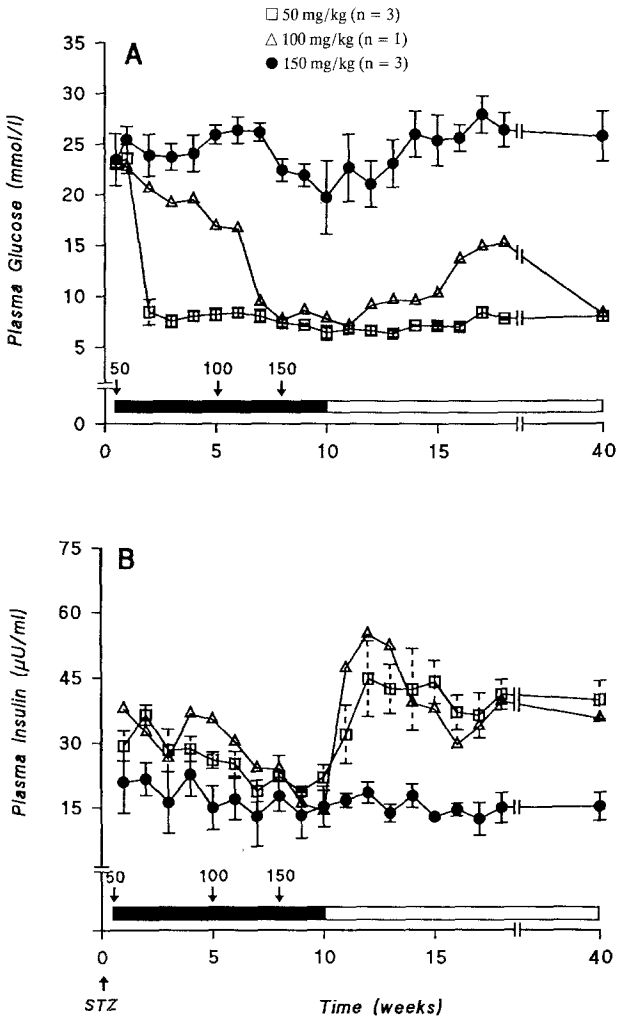

Fig 6. Plasma glucose (A) and insulin (B) in rats according to dose of naglivan at which glucose was normalized. Treatment was continued for 10 weeks (■), during which increasing doses of naglivan were administered, and was followed by withdrawal for an additional 30 weeks (□).

(DT-R 35.5 ± 1.9 *v* DT-NR 24.2 ± 3.6; μU/mL Fig 6B) at a time point when plasma glucose levels were reduced but had not yet reached control levels (DT-R 16.7 ± 2.2 *v* DT-NR 25.1 ± 1.7 mmol/L). Plasma insulin in DT-R was significantly reduced by naglivan to levels that were not different from DT-NR levels at 10 weeks (25.0 ± 3.6 μU/mL), whereas no further reduction was seen in DT-NR that had received the highest dose of 150 mg/kg/d. After treatment was withdrawn, plasma insulin levels in DT-R rebounded and remained significantly higher than in DT-NR, which showed no change in insulin levels. Animals that responded to a dose of 50 mg/kg/d maintained stable euglycemia, whereas one rat that had required 100 mg/kg/d showed a temporary reversion to mild hyperglycemia, which returned to normal at 30 weeks. The moderate reduction in glucose in DT-NR was reversed to pretreatment levels after treatment was withdrawn. At 30 weeks after withdrawal from naglivan treatment, DT-R had normal glucose tolerance as evidenced by the integrated glucose response, which was not significantly higher than in CT at OGTT 2 (data not shown). In parallel to what was earlier seen with LC and HC at OGTT 2, peak and total plasma insulin release in DT-R was significantly higher than in DT-NR. Insulin responses of DT-R and DT-NR were similar to those of LC and HC at OGTT 2, respectively.

DISCUSSION

From the correlative levels of plasma glucose and insulin in this study, it appears that STZ 55 mg/kg produced animals with a range of diabetes severity. The significantly lower insulin and higher glucose levels in HC relative to LC suggest that residual circulating insulin determined the relative requirement for vanadium. Because the comparatively lower insulin levels in HC were apparently offset by additional vanadyl intake, it appears that vanadium could act in a complementary or synergistic manner with the attenuated levels of endogenous insulin in diabetic animals. Similarly, in naglivan-treated animals, plasma insulin was significantly higher in DT-R relative to DT-NR. Interestingly, initial insulin levels in LC and DT-R were not significantly different (LC 36.0 ± 2.2 *v* DT-R 35.5 ± 1.9 μU/mL), whereas HC and DT-NR also showed similar values (HC 25.6 ± 3.3 *v* DT-NR 24.2 ± 3.6 μU/mL). Hence, it can be postulated that even higher doses of naglivan could have resulted in euglycemia in DT-NR. Several studies have reported the vanadium-induced reduction in plasma insulin in control animals, which supports the notion of an enhanced tissue sensitivity to endogenous insulin.[10,21] In this study, treatment of LC resulted in a decrease in plasma insulin to levels that were not different from HC levels at 10 weeks, which suggests a similar reduction in insulin demand in the long term. The successful decrease of plasma glucose in naglivan-treated DT-R was also accompanied by a downward trend in plasma insulin, which was not different from DT-NR levels by 10 weeks. There appeared to be a minimum requirement for a basal level of plasma insulin (~20 to 25 μU/mL) in the fed state, since there was no further decrease of insulin in rats treated with higher doses, and vanadyl treatment rarely reduced plasma insulin beyond this level. The trend toward higher plasma vanadium with lower plasma insulin at 10 weeks of treatment suggests that vanadium may have supplemented the remaining available circulating insulin.

Although the phenomenon of postwithdrawal euglycemia was first reported in animals that had begun treatment at 3 days after STZ injection and for 13 weeks after withdrawal from vanadyl,[18,19] the occurrence of prolonged euglycemia was presently observed in diabetic animals that had started treatment as late as 17 days after STZ injection, and has been extended to 20 to 30 weeks after withdrawal from treatment. The state of maintained euglycemia in diabetic animals after withdrawal from vanadyl sulfate was accompanied by a continuous increase in plasma insulin levels over 20 weeks. This phenomenon occurred primarily in LC that had significantly more insulin before treatment, and suggested that the remaining functional β cells were sufficient to respond to a higher insulin demand when vanadium was no longer being consumed. In CT, there was

a dramatic increase in circulating insulin, which reached 100 μU/mL at 20 weeks. It could be argued that the higher insulin level in CT is attributed in part to increased insulin resistance, which has been reported in rats with increasing age and weight.[24] However, the age and weight of CT were not different from those of LC at this time. Similarly, plasma insulin levels in naglivan-treated animals were higher by the first week after withdrawal from treatment. Overall, circulating insulin appeared to influence glucose levels after vanadyl treatment was withdrawn. The persistence of hyperglycemia in naglivan-treated DT-NR was associated with hypoinsulinemia, which was unimproved after discontinuation of treatment. In addition, the observed transitory period of normoglycemia after treatment withdrawal was accompanied by a gradual increase in plasma insulin levels to 50 to 80 μU/mL, which consistently declined to prewithdrawal levels concurrent with the return to hyperglycemia. This reversal occurred primarily in HC, which had been notably more insulinopenic than LC before treatment. Thus, the apparent inability of the pancreas to release greater amounts of insulin despite a larger insulin demand is consistent with the notion of a gradual exhaustion of limited insulin stores.[25]

The degree of glucose tolerance and insulin response during an OGTT reflected the prevailing status of animals during the fed state. Thus, at 3 weeks, when fed glucose levels were normal in virtually all diabetic groups, only a slight impairment in glucose tolerance was observed. However, in accordance with glycemic status at 20 weeks, a greatly impaired glucose tolerance was observed in rats that had reverted to hyperglycemia and was most distinct in [1.50]. Similarly, insulin responses in the various groups correlated with fed insulin values. Hence, coincident with the elevated levels of circulating insulin in CT and LC at 20 weeks, insulin responses in CT and LC were increased to threefold and twofold HC responses, respectively. As expected, insulin response was not significantly altered in HC at any time point from OGTT 0. The degree of glucose tolerance was observed to be independent of insulin response at 3 weeks after withdrawal from vanadyl (ie, there was no correlation between total glucose and insulin response). Notably, glucose tolerance was similar in HC and LC at 3 weeks despite the comparatively diminished insulin response in HC relative to LC, which suggests persistence of an even more enhanced insulin sensitivity in HC at 3 weeks after vanadyl was ceased. It is possible that vanadium is still available from several storage sites at a relatively short (3 weeks) period of withdrawal,[26] although it is undetectable in plasma.[27] At 20 weeks after withdrawal, the relative degree of glucose intolerance was proportionate to the concurrent insulin response to oral glucose, which suggests that the insulin released determined to a large extent the degree of glucose tolerance by this time. However, at this time euglycemia was still maintained in LC despite insulin levels that remained at 50% of CT levels in the fed state and at peak response to glucose. Thus, although it appears that the prolonged state of increased insulin sensitivity as a result of vanadium treatment that was evident at 3 weeks after withdrawal from treatment was still present to some extent at 20 weeks, at this prolonged period an improved insulin-secretory capacity may have played a more important role in maintenance of euglycemia.

In conclusion, it appeared that in a hypoinsulinemic state, effects of relative levels of residual insulin can be augmented by increasing doses of vanadyl, which may act in a complementary or synergistic manner with endogenous circulating insulin. Previous studies have presented evidence that supports the notion of a mechanism of vanadium in increasing tissue sensitivity to exogenously administered insulin. For instance, using hyperinsulinemic-euglycemic clamps, it was demonstrated that vanadium enhanced hepatic and muscle insulin sensitivity in STZ-diabetic rats.[28] In addition, vanadium administered to control[22] and STZ-diabetic[23] animals increased the hypoglycemic response to exogenous insulin. Moreover, naglivan (50 mg/kg/d) reduced the insulin requirement by half in untreated STZ-diabetic rats.[11] Furthermore, in spontaneously diabetic (BB) rats, vanadium reduced the amount of exogenous insulin required to maintain an aglycosuric state.[17] From the current study, it appears that the relative efficacy of vanadium in the whole animal may in fact depend on the presence of insulin, and that differences in degree of severity of the diabetic state and relative deficiency in residual circulating insulin can ultimately determine responsiveness to vanadium treatment. With regard to the postwithdrawal effects of vanadyl, it appeared that the sustained normal glucose homeostasis in the short term may have resulted from a continued enhancement in insulin sensitivity, whereas in the long term this effect was more closely associated with an improved pancreatic function. It is possible that long-term elimination of hyperglycemia by vanadium may have resulted in preservation of some β-cell function, since elevated glucose levels have been reported to damage β-cell integrity further.[29,30] Indeed, it has been suggested that vanadium, through a reduction in insulin demand, prevents exhaustion of insulin stores.[31] Altogether, results of this study confirm that improvements in the diabetic state may be extended over a prolonged period after treatment with either vanadyl sulfate or naglivan is ended. This is an apparently unique property that may be significant in the search for newer agents in diabetic therapy.

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