Partial Preservation of Pancreatic β-Cells by Vanadium: Evidence for Long-Term Amelioration of Diabetes

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Streptozotocin (STZ)-diabetic rats treated with vanadium can remain euglycemic for up to 20 weeks following withdrawal from vanadium treatment. In this study, we examined the effects of short-term vanadium treatment in preventing or reversing the STZ-induced diabetic state. Male Wistar rats were untreated (D) or treated (DT) with vanadyl sulfate for 1 week before administering STZ. Treatment was subsequently maintained for 3 days (DT3) or 14 days (DT14) post-STZ, after which vanadium was withdrawn. At 4 to 5 weeks post-STZ and following long-term withdrawal from vanadium, DT14 rats demonstrated levels of food and fluid intake and glucose tolerance that were not significantly different from those of age-matched untreated nondiabetic rats, and had significantly reduced glycemic levels in the fed state compared with D and DT3 groups. The proportion of animals that were euglycemic (fed plasma glucose < 9.0 mmol/L) was significant in DT14 (five of 10) relative to D (one of 10) and DT3 (one of 10) ($P = .01$). All euglycemic animals had an improved pancreatic insulin content that, albeit low (12% of control), was strongly linked to euglycemia in the fed state ($r = -.91$, $P < .0001$). Moreover, the highly significant correlation persisted with the analysis of untreated STZ-rats alone ($r = -.95$, $P < .0001$). Similarly, improvements in glucose tolerance and insulin secretory function in euglycemic rats were strongly correlated with small changes in residual insulin content. Hence, as vanadium pretreatment did not prevent STZ-induced β-cytotoxicity, the vanadium-induced amelioration of the diabetic state appears to be secondary to the preservation of a functional portion of pancreatic β cells that initially survived STZ toxicity. The partial preservation of pancreatic β cells, albeit small in proportion to the total insulin store, was both critical and sufficient for a long-term reversal of the diabetic state. These results suggest that apparently modest effects in preserving residual pancreatic insulin content can have profound consequences on glucose homeostasis and may bear important implications for interventions that have "limited" protective effects on β cells.

THE ANTIDIABETIC EFFECTS of vanadium salts have been extensively reported.[1,2] In addition to having potent glucose-lowering effects, vanadium has been demonstrated to prevent long-term secondary complications in streptozotocin (STZ)-diabetic rats.[3-5] Some mechanisms proposed for the glucose-lowering effects of vanadium in diabetic animals include an enhanced insulin sensitivity,[6] glucose uptake,[7,8] and expression of enzymes involved with glucose metabolism in liver[9] and muscle.[10] Some of the effects of vanadium in diabetic rats, such as correction of insulin resistance[11] and of defects in enzyme activity and/or expression,[12] have also been suggested to be secondary to the prevention of hyperglycemia. In addition, vanadium treatment was demonstrated to improve pancreatic insulin content in STZ-diabetic rats.[11,13] In these studies, residual insulin reserves in diabetic rats following vanadium treatment were 5.6-fold[13] to 7.8-fold[11] higher than in untreated diabetic animals. However, despite these improvements overall changes in the insulin store were noted to be relatively insubstantial when viewed as a percentage of control levels, and were not considered an important factor in the regulation of normal glucose homeostasis.[11,13]

One interesting phenomenon that is not completely understood is a continued absence of diabetic symptoms in STZ-diabetic rats for up to 20 weeks following withdrawal from vanadium treatment.[14,15] These animals appeared to maintain a chronic euglycemic state despite only minor improvements in pancreatic secretory function, amounting to approximately 12% of control.[16] Hence, it was thought that these animals had sustained an increased sensitivity to circulating insulin after vanadium treatment was withdrawn, as it was possible that tissue vanadium stores could be released and continue to exert insulin-mimetic effects. However, we further hypothesized that a partial preservation of the insulin secretory function and/or insulin store may play a role in the maintenance of euglycemia following long-term withdrawal from vanadium treatment.

Recent studies have suggested that a reduction in insulin biosynthesis and secretion can render β cells less susceptible to cytotoxic events.[17,18] In support of this, prophylactic insulin treatment prevented the onset of diabetes in genetically susceptible BB rats[19] and NOD mice.[20] Conversely, high glucose potentiated the diabetogenic effects of STZ in vitro[21] and in vivo.[22] Since vanadium treatment has been demonstrated to reduce insulin secretion and circulating insulin levels in control animals,[3,5] we also hypothesized that vanadium could potentially prevent the onset of STZ-diabetes. Thus, our aim in this study was to determine whether vanadium treatment administered before STZ injection can protect β cells from STZ toxicity and/or preserve residual β cells when the treatment is continued for a short time after STZ. In addition, we considered whether the long-term reversal of the diabetic state by vanadium could be secondary to an effect on the residual pancreatic insulin store.

MATERIALS AND METHODS

Treatment and Maintenance of Animals

Male Wistar rats (160 to 190 g) aged 5 to 6 weeks were obtained from Charles River (St. Constante, Quebec, Canada). Rats were divided into three nondiabetic groups (one untreated [C], n = 7, and two treated

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[CT3, n = 7, and CT14, n = 7]) and three STZ-diabetic groups (one untreated [D], n = 10, and two treated [DT3, n = 10, and DT14, n = 10]). Vanadyl sulfate ($VOSO_4 \cdot 3H_2O$; Fisher Scientific, Fair Lawn, NJ) was administered at a concentration of 0.75 mg/mL in the drinking water first to the control and STZ-treated groups for 3 days, followed by 1.00 mg/mL for 4 days. At 7 days, STZ (55 mg/kg intravenously; Sigma, St Louis, MO) was administered to the STZ groups (D, DT3, and DT14) while control groups received vehicle (NaCl 154 mmol/L, pH 7.2). Vanadium treatment was continued at the same concentration for 3 days in CT3 and DT3, and for 14 days in CT14 and DT14. The concentration (1.0 mg/mL) of vanadyl sulfate was chosen on the basis of previous studies showing that administration of this dose for 10 weeks to rats made diabetic with STZ 55 mg/kg resulted in long-term amelioration of diabetic symptoms for up to 20 weeks following withdrawal of vanadium treatment.[15] Plasma glucose and insulin levels, body weight, and food and fluid intake were monitored frequently during and after treatment. The experiment was terminated at 5 weeks following STZ injection, and blood was obtained from the nicked tail vein into heparinized glass capillary tubes and immediately centrifuged to obtain plasma. Determination of plasma glucose, urea nitrogen, creatinine, and aspartate aminotransferase was made using kits from Boehringer (Mannheim, Germany). Plasma insulin level was measured via radioimmunoassay using rat insulin standards (Novo Research Institute, Copenhagen, Denmark). The radioimmunoassay allows for measurement of plasma insulin or pancreatic extracts using volumes of 25 μL with an interassay and intraassay coefficient of variation less than 10% and is sensitive to 7 μU/mL.[23]

Oral Glucose Tolerance Test

At 4 weeks after STZ, overnight-fasted rats were lightly anesthetized with sodium pentobarbital 20 mg/kg intraperitoneally. In our experience, this does not affect the kinetics of either plasma glucose or insulin during an oral glucose tolerance test (OGTT). Rats were administered glucose (1 g/kg of a 40% glucose solution) by oral gavage. Blood samples (350 μL) were collected before (time 0) and at 10, 20, 30, and 60 minutes after the glucose dose from the nicked tail vein. Plasma was stored frozen at −70°C for measurement of insulin and glucose levels. The areas under the curve (AUCs) for glucose and insulin over 60 minutes were calculated to quantify the relative degrees of glucose tolerance (AUC_g) and insulin response (AUC_i) to an oral glucose load.

Subclassification of STZ Animals According to Glycemic Status

At the end of the experiment, it was found that there was a wide range in the glycemic status of STZ rats. Thus, to distinguish between the glycemic response to vanadyl treatment and the inherent differences within the various groups to the diabetogenic effects of STZ, animals from D, DT3, and DT14 groups were pooled (n = 30) and retrospectively classified according to fed glycemic levels and glucose tolerance. This resulted in three subgroups: euglycemic and near-normal glucose-tolerant ([E] n = 7), hyperglycemic and near-normal glucose-tolerant ([H + GT] n = 13), and hyperglycemic and glucose-intolerant ([H + GI] n = 10). Diagnostic criteria pertaining to each subclassification are described in the results.

Pancreatic Insulin Extraction

At termination, pancreata were dissected, cleared of lymph nodes and fat, blotted, and weighed. The pancreas was immediately homogenized using a polytron homogenizer (position 5) in 5 mL cold 2N acetic acid for 5 seconds, and boiled at 100°C for 10 minutes. The extract was centrifuged at 15,000 rpm for 10 minutes at 4°C. The resulting supernatant was frozen in liquid nitrogen and stored at −70°C until analysis of insulin.

Histological Analysis

A portion of the pancreas was fixed in 2% Formalin for 1 to 2 days, dried, and embedded in paraffin. Sections were stained for granulated β cells by the modified aldehyde fuchsin method as previously described,[24] and examined by light microscopy.

Vanadium Levels

At termination, samples of tissue from kidney, liver, muscle, and bone were analyzed for vanadium levels via atomic absorption using a method previously described.[25] The detection limit of this procedure has been reported at 0.2 nmol/mL blood or 0.05 nmol/g tissue dissolved in concentrated nitric acid:water solutions.[26]

Statistical Analysis

One- or two-way ANOVA was used, as appropriate, followed by the Newman-Keuls test. Fisher's exact test was used to determine differences in the proportion of STZ rats between glycemic subgroups. P less than .05 was considered significant. Data are expressed as the mean ±SEM.

RESULTS

General Characteristics of STZ Rats Treated With Vanadium

Vanadium treatment reduced daily food intake by 13% (22.6 ± 0.4 *v* 25.9 ± 0.4 g/rat) and fluid intake by 36% (29.8 ± 0.2 *v* 46.7 ± 1.7 mL/rat) before STZ. Food intake was not significantly elevated in the rats at 3 days after STZ, but at 2 weeks it was 33% greater than in controls. The self-administered dose of vanadium before withdrawal was similar between groups (CT3, 0.55 ± 0.05 mmol/kg/d; CT14, 0.54 ± 0.06; DT3, 0.56 ± 0.03; DT14, 0.57 ± 0.04). On withdrawal of vanadium, food and fluid intake in DT3 animals returned to levels similar to those in D rats, although these parameters in DT14 were not different from control levels (Table 1). Body weight was reduced by treatment with vanadium for 1 week before STZ (data not shown). Vanadium intake for 2 weeks post-STZ also reduced weight gain significantly in DT14 (by 10%) relative to D. After long-term withdrawal from vanadium treatment at 5 weeks after STZ, weight gain in all STZ groups was similar and lower than in controls (Table 1).

Vanadium treatment for 1 week before STZ injection was found to reduce plasma insulin levels by at least 40% (DT3, 16.9 ± 2.6 μU/mL; DT14, 18.5 ± 2.5; D, 30.6 ± 3.8; $P < .05$), but had no effect on glycemia. Mean plasma glucose in D after STZ was 15.5 ± 1.9 mmol/L (day 1) and 20.2 ± 0.9 mmol/L (day 2), and was reduced significantly and to the same extent by vanadium treatment in both groups ($P < .05$). After long-term withdrawal from vanadium treatment at 5 weeks after STZ, both D and DT3 demonstrated marked hyperglycemia, whereas mean glycemia in DT14 was significantly reduced ($P < .03$; Table 1). Although mean plasma insulin levels were not significantly different between STZ (untreated and treated) groups, they remained significantly lower than in controls.

At 4 weeks after STZ, vanadium treatment had been withdrawn from CT3 and DT3 for 3.5 weeks and from CT14 and DT14 for 2 weeks. The integrated glucose AUC over 60 minutes (AUC_g) was significantly greater in D and DT3 relative to controls ($P < .05$), whereas the mean AUC_g of DT14 was not significantly different from that of either C or D. The integrated insulin response (AUC_i) was significantly lower in CT14 relative to controls ($P < .05$). All STZ groups had mean AUC_i

Table 1. Characteristics of C and STZ Groups at Weeks 4 to 5

Characteristic	C (n = 7)	CT3 (n = 7)	CT14 (n = 7)	D (n = 10)	DT3 (n = 10)	DT14 (n = 10)
Vanadyl treatment						
Pre-STZ (d)	0	7	7	0	7	7
Post-STZ (d)	0	3	14	0	3	14
Body weight gain (g)	157 ± 6	171 ± 3	162 ± 5	115 ± 8*	104 ± 8*	126 ± 8*
Food intake (g/d)	28 ± 1	29 ± 1	31 ± 1	40 ± 1*	45 ± 4*	35 ± 1†
Fluid intake (mL/d)	56 ± 4	59 ± 4	64 ± 5	126 ± 12*	168 ± 17*	89 ± 14†
Plasma glucose (mmol/L)	6.2 ± 0.2	6.5 ± 0.1	6.3 ± 0.1	18.2 ± 1.7*	20.0 ± 1.6*	12.8 ± 2.0†
Plasma insulin (μU/mL)	50 ± 4	53 ± 5	42 ± 6	33 ± 4*	23 ± 4*	35 ± 6*
AUC_g (mmol/L · min)	445 ± 14	439 ± 16	444 ± 17	767 ± 89*	805 ± 93*	620 ± 51
AUC_i (μU/mL · min)	2,612 ± 426	2,448 ± 327	1,727 ± 155*	1,132 ± 114*	1,088 ± 115*	1,161 ± 116*

NOTE. Results are the mean ± SEM.
*$P < .05$ *v* C.
†$P < .05$ *v* D.

values approximately 40% of control values and not different from one another.

At termination, vanadium was not detectable in the plasma of all untreated or treated rats. Vanadium levels in bone and kidney of untreated rats were undetectable, and were higher in DT14 (bone, 90.3 ± 5.9; kidney, 5.9 ± 0.6 nmol/g) than in DT3 (bone, 70.7 ± 7.9; kidney, 4.7 ± 0.6 nmol/g). These levels are lower than those reported in diabetic rats after long-term (5-week) treatment[26] or after a 16-week withdrawal period following a 1-year treatment.[27] Vanadium levels in the pancreas and liver were detectable but low (<2.0 nmol/g). There were no differences in plasma urea nitrogen, creatinine, or aspartate aminotransferase between control and STZ groups. At a fixed concentration of vanadium (1.0 mg/mL) in the drinking water, there was no incidence of diarrhea.

Persistent Normoglycemia in STZ Rats Following Withdrawal From Vanadium

When individual rats were examined, chronic hyperglycemia was found in nine of 10 animals in D, with one rat remaining euglycemic (plasma glucose < 9.0 mmol/L) by the end of the study (Fig 1A). A similar proportion (one of 10) of animals was found to be euglycemic in DT3 (Fig 1B). However, extending vanadium treatment for 2 weeks after STZ corrected plasma glucose in 50% (five of 10) of the rats, which further maintained euglycemia for 3 weeks after treatment was withdrawn (Fig 1C). In the remaining five rats in DT14, there was an intermediate reduction in glucose levels during vanadium treatment, and severe hyperglycemia recurred after withdrawal from treatment. Five of 10 euglycemic rats in DT14 had significantly lower glucose levels by day 2 after STZ relative to rats in this group that had recurrent hyperglycemia after treatment was withdrawn (14.1 ± 0.9 *v* 18.2 ± 0.3 mmol/L, respectively, $P < .05$).

Pancreatic Insulin Content

There was no difference in pancreatic weight in the various groups. Vanadium treatment did not alter pancreatic insulin content in control animals (Fig 2). At 5 weeks after STZ, there was a marked reduction of at least 90% for the mean insulin content in all STZ groups. Pancreatic insulin content in DT14 was significantly greater than in DT3 ($P < .05$) but not different versus D.

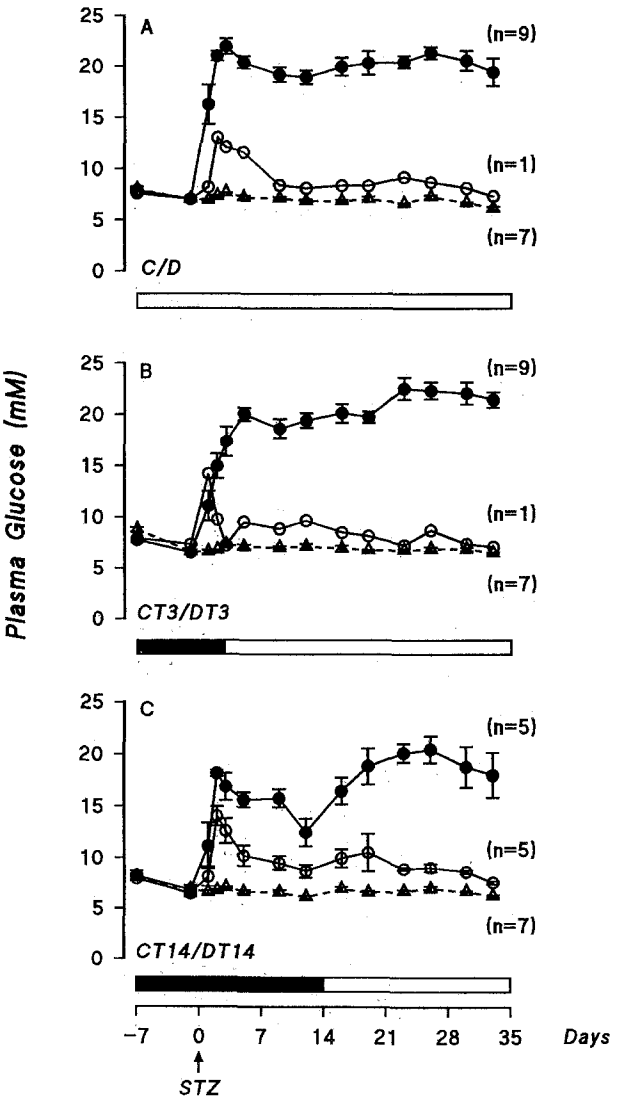

Fig 1. Effects of vanadium treatment followed by withdrawal period on fed plasma glucose in STZ-diabetic animals (○, E; ●, hyperglycemic) and respective controls (△): (A) C/D, (B) CT3/DT3, and (C) CT14/DT14. (■) Vanadyl treatment. Results are the mean ± SEM.

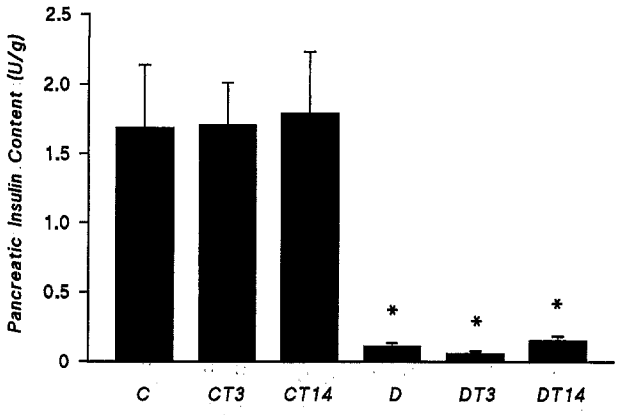

Fig 2. Pancreatic insulin content in control and STZ-diabetic groups at 5 weeks post-STZ (*$P < .05$ v C). Results are the mean ± SEM.

Classification of STZ Animals

As previously reported using a dose of STZ 55 mg/kg,[15] we observed a highly heterogeneous population of animals. This added a degree of complexity to measuring the actual glycemic response to vanadium treatment. Hence, to distinguish between the progressive stages of severity in the diabetic state and the glucose-lowering effects of vanadium per se, all STZ (untreated and treated, n = 30) rats were pooled and classified according to glycemic status (Table 2). This was accomplished using plasma glucose levels in the fed state and in the acute response to an oral glucose challenge in fasted animals. There was a threefold range in fed plasma glucose levels among STZ rats (7.0 to 24.7 mmol/L). Hence, STZ rats were considered euglycemic (E) when the fed plasma glucose level was less than 9.0 mmol/L. Mean glycemia in the E group (n = 7) was 7.5 ± 0.1 mmol/L. In addition, there was a considerable range (fourfold) in the degree of glucose tolerance, and the AUC_g calculated for the first 60 minutes of a glucose tolerance test was 451 to 1,742 mmol/L · min. It was found that the E subgroup also demonstrated a near-normal glucose tolerance, as only mean glycemia at the 60-minute time point was significantly greater than the control (Fig 3A). Accordingly, the mean AUC_g (562 ± 89 mmol/L · min) in the E subgroup was not significantly different from that in the nondiabetic control (inset). It was also observed that some of the STZ rats that were hyperglycemic (plasma glucose > 9.0 mmol/L, n = 23) showed near-normal glucose tolerance (H + GT). Hence, the hyperglycemic animals in which AUC_g was found to be within ±2 SD of the mean for the E group (385 to 739 mmol/L · min) were classified as H + GT (n = 10). Alternately, the remaining hyperglycemic rats with levels above this AUC_g range were classified as hyperglycemic/glucose-intolerant ([H + GI] n = 13). Thus, whereas the mean AUC_g in H + GT rats was not different from the control level, it was significantly greater in H + GI (Fig 3A, inset). The glucose-stimulated insulin response in STZ rats was significantly less than in the control, and there was a downward trend according to severity of the diabetic state (E > H + GT > H + GI, inset).

Table 2. Classification of STZ Animals According to Glycemic Status

Parameter	Group		
	E	H + GT	H + GI
Fed plasma glucose (mmol/L)	<9.0	>9.0	>9.0
AUC_g (mmol/L · min)	<740	<740	>740
Treatment			
D (n = 10)	1	4	5
DT3 (n = 10)	1	5	4
DT14 (n = 10)	5*	4	1
Total (n = 30)	7	13	10

*Significantly different from D ($P = .01$).

The proportion of rats in each glycemic subcategory is shown in Table 2. The number of E rats was significant in DT14 (five of 10) compared with D (one of 10) and DT3 (one of 10) ($P = .01$). Moreover, the total number of animals (E and H + GT) that had near-normal glucose tolerance was higher in DT14 (nine of 10) relative to D (five of 10) ($P = .01$) or DT3 (six of 10) ($P = .03$). In addition, mean fed glycemia in H + GT (18.1 ± 1.1 mmol/L) was lower than in H + GI (22.3 ± 0.5 mmol/L, $P < .05$), whereas fed plasma insulin levels in E (37.9 ± 2.9 μU/mL) and H + GT (37.2 ± 3.6) were significantly lower than in the control, and were further reduced in H + GI (17.2 ± 2.9) ($P < .05$). Weight gain was significantly lower than in the control, but was highest in E (138 ± 6 g), followed by H + GT (119 ± 5 g), and least in H + GI (93 ± 6 g).

Correlations Between Residual Insulin Content and Glycemic Status

Figure 4A depicts pancreatic insulin content of STZ animals analyzed according to glycemic status. The E group had a mean insulin content of approximately 12% (0.20 ± 0.02 U/g) of the control level (1.69 ± 0.48 U/g), which was two and four times higher than for H + GT and H + GI, which had mean insulin contents of approximately 6% (0.10 ± 0.01 U/g) and 3% (0.05 ± 0.01 U/g), respectively. Since the severity of the diabetic state appeared to be associated with the relative insulin content, we further questioned the extent to which the variance in glycemic status among STZ animals could be linked to changes in residual insulin content. Interestingly, it was found that residual insulin content in the pooled STZ animals (n = 30) correlated very strongly with the threefold range in plasma glucose levels in the fed state ($r = -.91$, $P < .0001$; Fig 4B). Thus, H + GI rats that had the highest glucose levels (range, 19.5 to 24.7 mmol/L) had the lowest residual insulin stores (95th percentile range, 2% to 4% of control), whereas the E subgroup that had the highest residual insulin content (9% to 16%) had correspondingly lower glycemic levels (7.0 to 8.1 mmol/L). Intermediate between these groups, H + GT had glucose levels that varied from 10.2 to 23.3 mmol/L and a range in insulin content of 5% to 10%. Notably, this strong correlation persisted with the analysis of untreated STZ rats alone ($r = -.95$, $P < .0001$; Fig 4C).

Residual insulin content in the pooled STZ rats (n = 30) was highly correlated with the fourfold range in glucose tolerance in a negative, hyperbolic manner (AUC_g, $r = -.84$, $P < .0001$; Fig 5A) and with a fourfold range in insulin response in a positive manner (AUC_i, $r = .70$, $P < .0001$; Fig 5B). Both correlations persisted with the analysis of untreated STZ rats alone (insets). Glucose intolerance (according to these criteria) was observed only below an insulin content of approximately

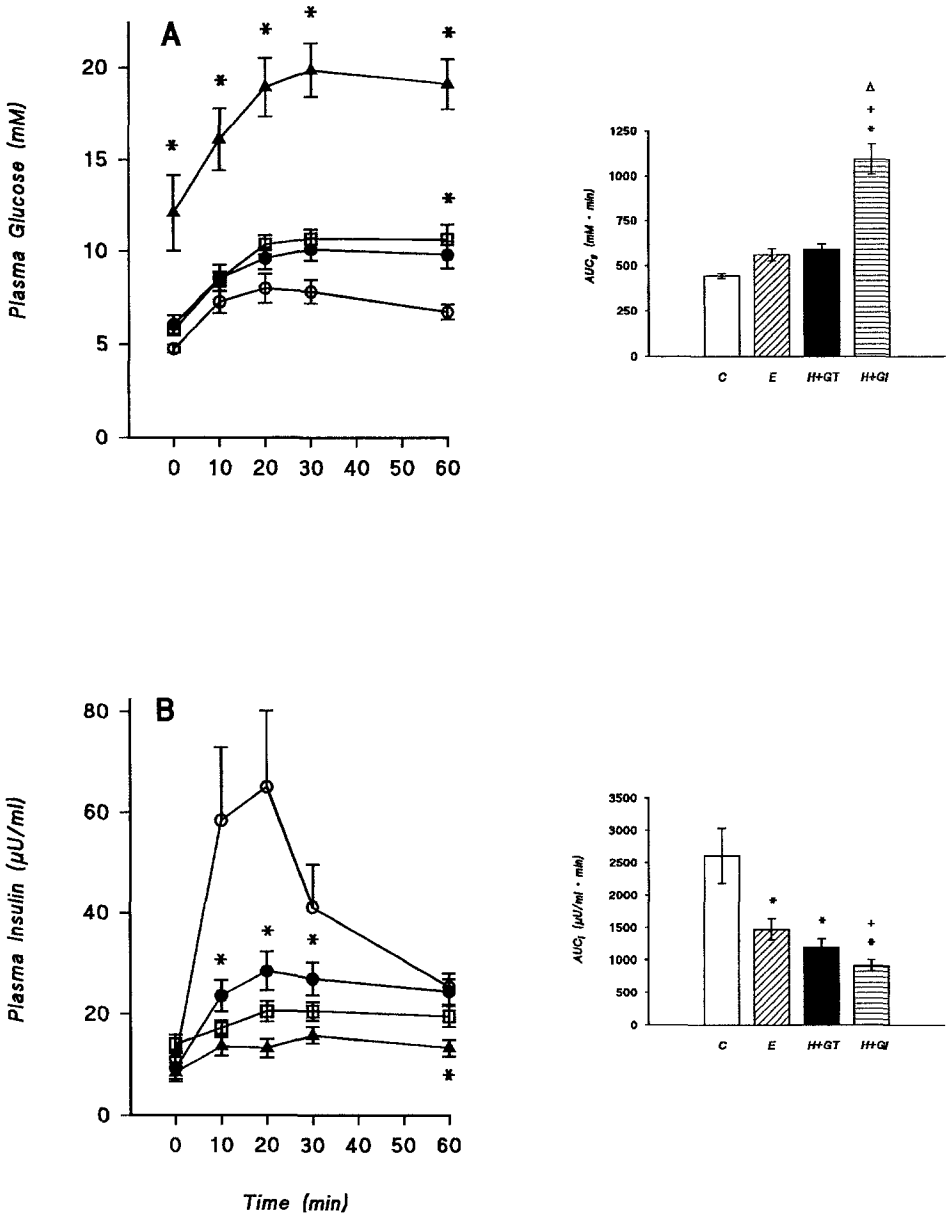

Fig 3. Differences in glucose tolerance and insulin response in pooled diabetic animals (untreated and vanadyl-treated) during an OGTT at 4 weeks post-STZ. Plasma glucose (A) and insulin (B) response to an OGTT in STZ rats: ●, E; □, H + GT; ▲, H + GI; and ○, untreated control. Bar graphs represent the AUC_g (C) and AUC_I (D) response of control and diabetic rats according to glycemic status. *$P <$ .05 *v* C; $^{+}P <$ .05 *v* E; $^{\triangle}P <$ .05 *v* H + GT. E, n = 7; H + GT, n = 13; H + GI, n = 10; C, n = 7. Results are the mean ± SEM.

100 mU/g, or 6% of the control level (Fig 5A). The residual insulin store was also correlated with fed plasma insulin levels (r = .54, P = .006) and body weight (r = .53, P = .004) (data not shown).

Histological Examination of β Cells

To ascertain if changes in pancreatic insulin content could be associated with an altered number of histologically detectable β cells, pancreatic sections were stained, and the number of granulated β cells was counted in five similarly sized islets per section. In control rats (untreated and treated), several islets were found to contain a substantial number of well-granulated β cells, indicated by the darkly stained areas within each islet (Fig 6A). In contrast, the number of granulated β cells per islet was markedly depleted in H + GT (4.7 ± 0.8, not shown) and almost undetectable in H + GI (1.1 ± 0.4; Fig 6B). However, in sections taken from E rats, each islet contained a significant number of darkly stained β cells (16.8 ± 0.6; Fig 6C). The number of histologically detectable β cells per islet was found to be consistent in at least 25 islets per group.

DISCUSSION

We questioned whether short-term vanadium treatment could improve pancreatic insulin content and function, and provide an explanation for a long-term amelioration of the diabetic state after treatment withdrawal. Animals were treated with vanadium for 1 week before STZ to ascertain any protective effects of vanadium against STZ-induced β-cytotoxicity, perhaps by decreasing insulin secretion in vivo. However, STZ-induced depletion of insulin stores in DT3 was not improved despite a reduction of plasma insulin levels by 40%. Alternatively, at 3 weeks after withdrawal from short-term (2-week) treatment following STZ, glucose levels in DT14 remained near normal, suggesting that the postwithdrawal maintenance of glucose

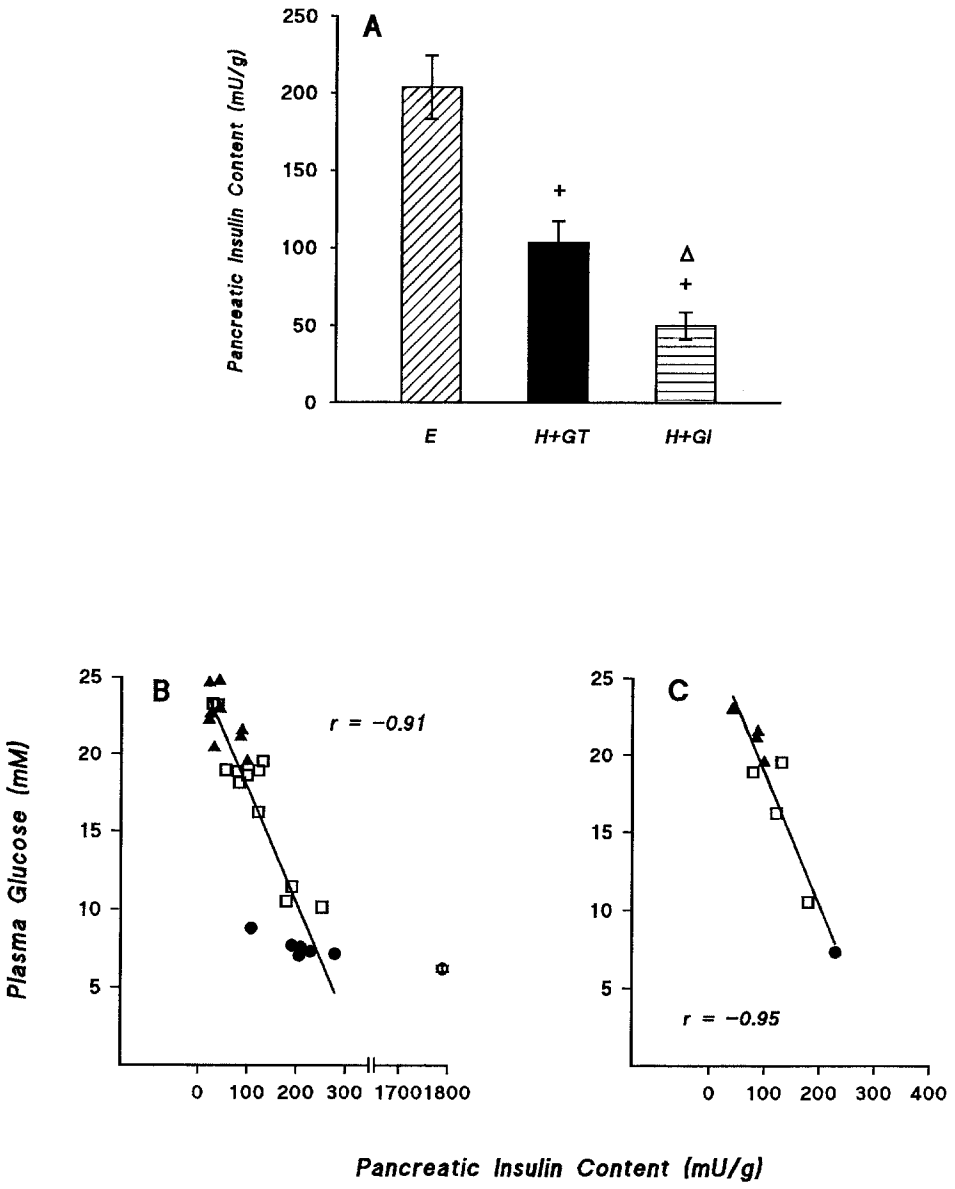

Fig 4. Association between glycemic status and residual insulin content at 5 weeks post-STZ. (A) Pancreatic insulin content in STZ-diabetic animals according to glycemic status. $P < .05$ v E^+ or $H + GT^6$. Results are the mean ± SEM. Correlation plots are shown for plasma glucose levels in the fed state and pancreatic insulin content in pooled (untreated and treated) diabetic rats ($r = -.91$, $P < .0001$) (B) and in untreated diabetic rats alone ($r = -.95$, $P < .0001$) (C): ●, E; □, H + GT; ▲, H + GI; ○, mean value for untreated nondiabetic rats (n = 7).

homeostasis was probably due to the effects of vanadium treatment after administration of STZ. We further found that food restriction (unlike vanadium treatment) in diabetic rats did not induce normoglycemia in any of the pair-fed animals,[28] thus ruling out any beneficial effects of a reduced food intake per se, contrary to previous suggestions.[29]

We have reported that STZ (55 mg/kg) produces a variable severity of diabetes in rats.[15] A similar heterogeneity in the diabetic state has been described in spontaneously diabetic BB rats,[30] Chinese hamsters,[31] and early type I diabetes in humans.[32] To illustrate and distinguish between progressive stages of diabetes in this study, STZ rats were subclassified, although not according to human diagnostic criteria.[33] This classification scheme allowed us to distinguish between the varying degrees of STZ diabetes per se and the actual response to vanadium treatment. Using this method, we found that a greater number of DT14 animals were euglycemic (50%) and had near-normal glucose tolerance (90%) compared with D and DT3 groups. It should be noted that increasing the vanadyl concentration to 1.5 mg/mL increases the euglycemic response to 100%.[15]

Since vanadium can accumulate in tissue stores and potentially continue to exert effects,[15] it is possible that the glucose-lowering effects of vanadium after withdrawal from treatment in DT14 could have been influenced by the shorter duration of withdrawal (2 weeks) versus the longer period of withdrawal (3.5 weeks) for DT3. Indeed, the lower AUC_i in CT14 suggests continued enhancement of insulin action by vanadium at 2 weeks after withdrawal from treatment. However, we have previously reported that after long-term treatment with the same concentration of vanadyl sulfate (1 mg/mL), eight of nine animals remained euglycemic up to 20 weeks after withdrawal of treatment.[15] On the other hand, five of six animals that required 1.25 to 1.50 mg/mL vanadium to achieve normoglycemia were found to revert to hyperglycemia at various times after withdrawal from treatment.[15] At 20 weeks, it appeared that the persistence of normoglycemia following vanadium treatment

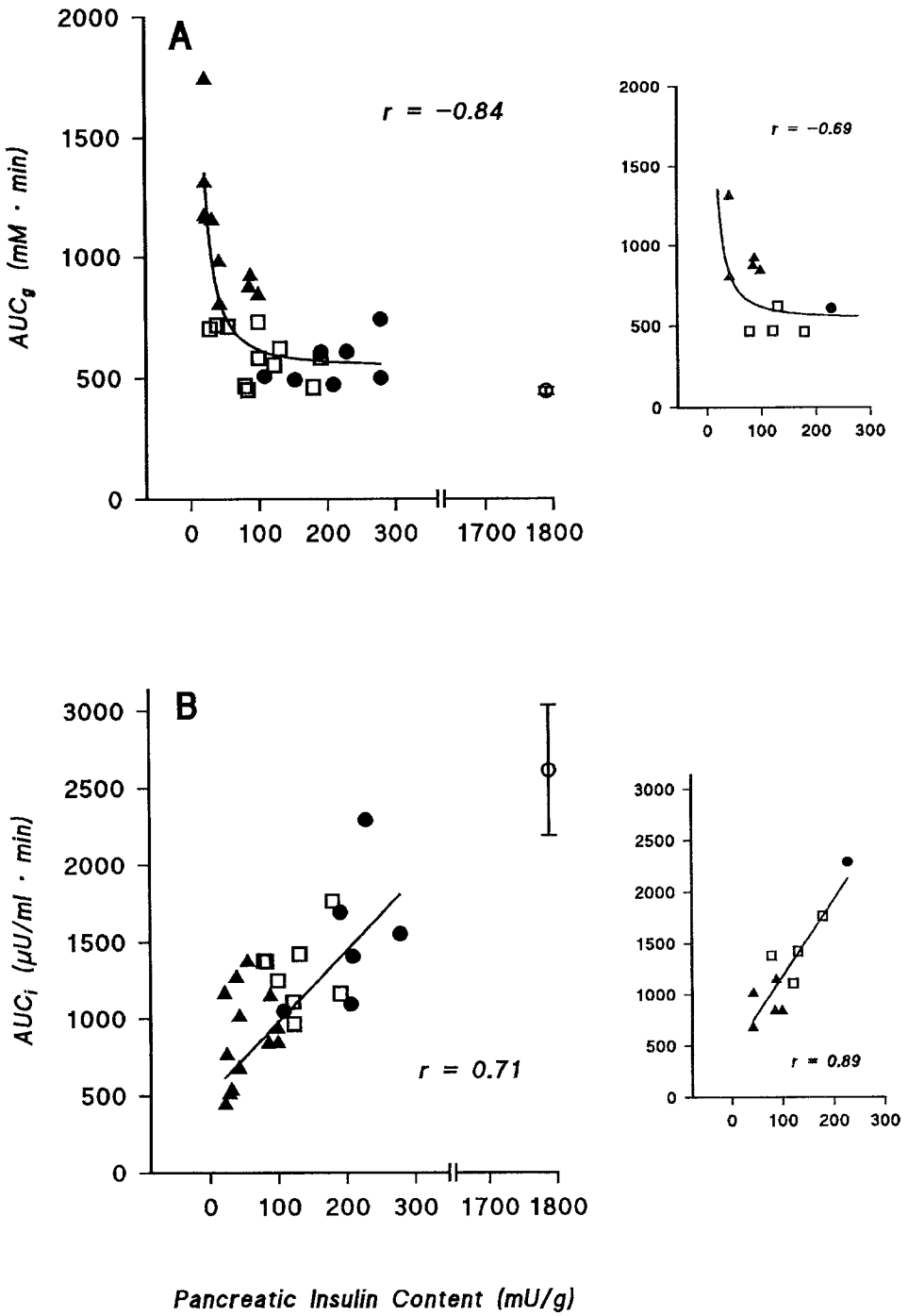

Fig 5. Association between glucose tolerance and insulin secretory function with residual insulin content. Correlation plots are between (A) AUC_g ($r = -.84$, $P < .0001$) for pooled diabetic rats and for untreated diabetic rats alone (inset) and (B) AUC_i ($r = .71$, $P < .0001$) during an OGTT at 4 weeks post-STZ and residual insulin content in diabetic rats: ●, E; □, H + GT; ▲, H + GI; ○, mean values for untreated control rats (n = 7).

was more likely due to an improved insulin secretory activity rather than to the continued effects of stored vanadium.[15] In this study, vanadium was detected in bone and kidney in STZ animals after treatment withdrawal and could potentially exert an effect. However, fed glycemia and glucose tolerance in the pooled STZ rats were strongly correlated with residual insulin content, and these correlations persisted on the analysis of untreated STZ rats alone, supporting the idea that near-normal glucose homeostasis in STZ rats was more likely dependent on insulin stores.

The finding that rats sustaining a markedly depleted insulin store can achieve euglycemia and near-normal glucose tolerance indicates that a modest (≤5%) improvement in the pancreatic insulin reserve is physiologically relevant and can induce a long-term amelioration of the diabetic state. Interestingly, residual pancreatic insulin reserves were increased by 5.6-fold[13] to 7.8-fold[11] in vanadium-treated diabetic rats versus untreated rats. However, in these studies, normal glucose homeostasis was not attributed to overall changes in the insulin store, which appeared to be relatively insubstantial when viewed as a percentage of control levels.[11,13] Interestingly, the pancreatic insulin content found in vanadium-treated rats (~185

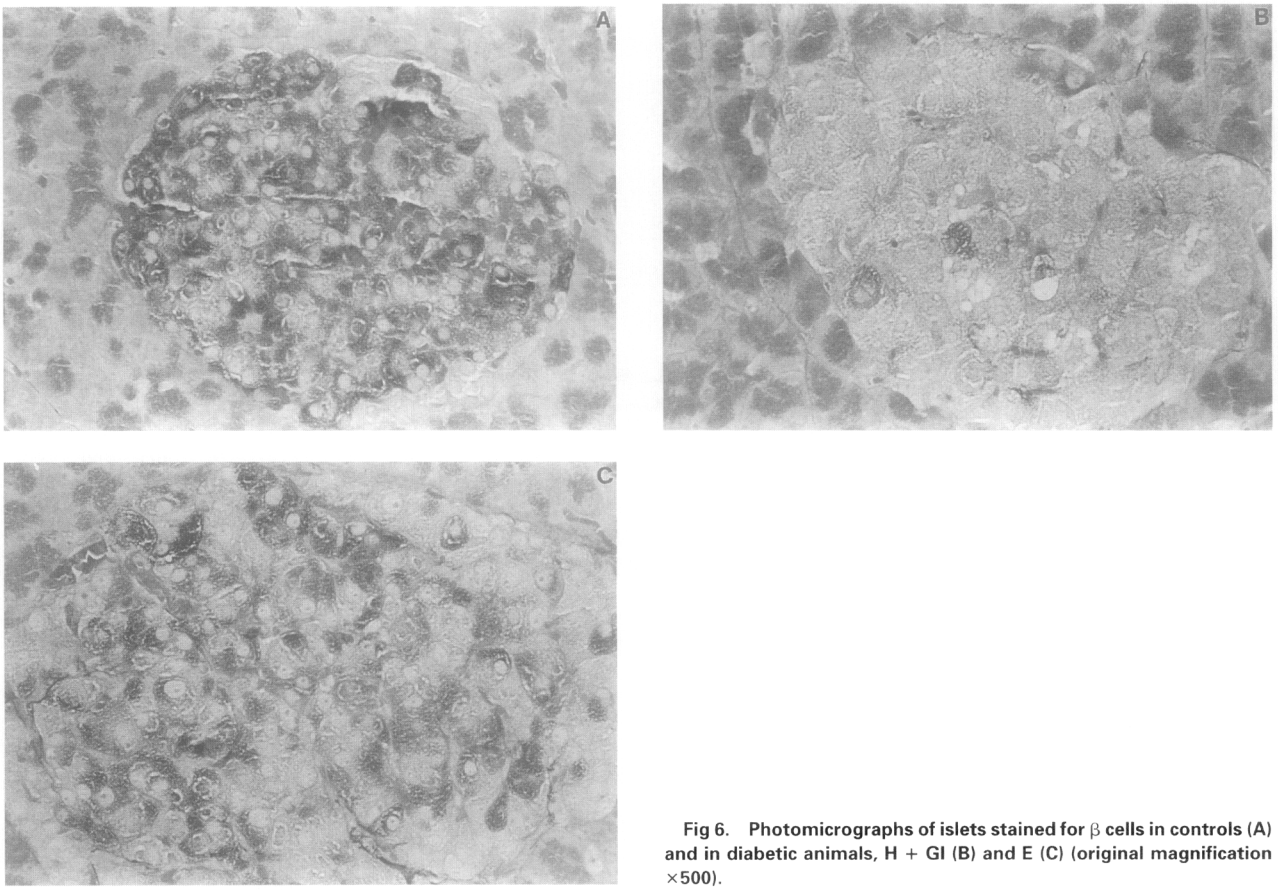

Fig 6. Photomicrographs of islets stained for β cells in controls (A) and in diabetic animals, H + GI (B) and E (C) (original magnification ×500).

mU/g[13]) is within the range (150 to 250 mU/g) presently found to support euglycemia per se. An even greater improvement in insulin reserve was found in diabetic rats in which insulin resistance was found to be reversed by vanadium treatment, amounting to approximately 33% of control levels.[11] Thus, it would appear that the relatively minor improvements in pancreatic insulin content rather than the direct effects of vanadium per se could have contributed to the overall amelioration of the diabetic state and may have adequately sustained long-term euglycemia after treatment withdrawal. In support, the current levels of the pancreatic insulin store (12%) in the E subgroup match the residual insulin secretory function in euglycemic diabetic animals following long-term withdrawal from vanadium.[16]

It has long been recognized that both humans[34] and rats[35] develop overt diabetes when at least 90% of the pancreas is removed, and that nonfasting hyperglycemia occurs in STZ-treated mice only when the reduction in islet function is greater than 90%.[36] In this study within a narrow range of residual pancreatic insulin (1% to 17% of control), the range in glycemic levels is considerable, from normal to severe hyperglycemia. In contrast, Junod et al[37] reported a correlation between glycemia (from high to normal) and the residual insulin store over a much wider range in insulin content (1% to 50% of control) when measured 24 hours after intravenous administration of STZ. However, insulin stores were further depleted over 28 days, and although no correlation was reported at that time, normoglycemia was evident with a residual insulin content of approximately 20%. Presently, despite the notion that several regulatory factors could simultaneously affect glucose homeostasis, the highly significant linear correlation between glycemia and the residual insulin store at 5 weeks post-STZ suggests that fed plasma glucose can be an accurate index of residual insulin content within the low range (<10% of control) during a stable, chronic STZ-diabetic state. Furthermore, it suggests that small changes in insulin content that may be easily overlooked could nevertheless be functionally significant. For instance, Le et al[38] reported significantly lower glycemia associated with an overall increase of approximately 2.3% of the total pancreatic insulin store in cholesterol-pretreated STZ-diabetic mice. Similarly, Serradas et al[39] found an improved β-cell function associated with an increased (~10%) insulin reserve in gliclazide-treated STZ-diabetic rats. Since these increases in insulin content were statistically insignificant in both studies, their contribution to the overall improvement in the diabetic state was not considered. Thus, in studies using STZ-diabetic models, it appears that a more detailed analysis of the diabetic groups may be required to reveal associations between overall improvements in the diabetic state and modest changes in the insulin store.

Although the current levels of pancreatic insulin content (12%) in E rats were found to match the residual secretory function (12%) in vanadium-withdrawn rats,[16] they are in contrast to the islet insulin area estimated by immunohistochemistry (IHC) (85%).[16] Two studies have compared pancreatic

insulin reserve and β-cell mass and function in the same animal in conditions of STZ diabetes.[40,41] Pancreatic insulin content in STZ-diabetic mice correlated highly with and was quantitatively equivalent to β-cell function and mass determined via dithizone staining.[40] Similarly, residual insulin content in STZ-diabetic baboons correlated linearly with pancreatic function ($r = .92$).[41] However, β-cell mass detected via IHC in diabetic baboons was 40% to 50% of control levels when both pancreatic secretory function and the insulin store were nonexistent. Conversely, IHC was found to underestimate the residual β-cell number in STZ-diabetic rats, relative to in situ hybridization.[42] Hence, in the unique case of STZ diabetes wherein residual β cells are severely degranulated, pancreatic insulin content rather than IHC-determined β-cell mass may be a more quantitative correlate to pancreatic secretory function. Indeed, in this study, in vivo insulin secretory function (AUC_i) was highly correlated with the residual insulin store.

One plausible mechanism of β-cell preservation is that the removal of hyperglycemia by vanadium reduced glucotoxicity and the subsequent functional and structural exhaustion of β cells. Indeed, early treatment of human diabetes with insulin has long-term beneficial effects on β cells.[43] Moreover, short-term insulin treatment following induction of STZ diabetes has been found to reverse the diabetic state for an indefinite period.[44,45] Diazoxide, an inhibitor of insulin secretion, decreased the incidence of diabetes in BB rats[46] and improved β-cell glucose responsiveness and insulin content in pancreatectomized rats,[47] supporting the notion that excessive insulin secretion by a compromised β-cell mass leads to exhaustion.[48] Because vanadium treatment before STZ did not prevent or minimize the STZ-induced loss of β-cell insulin content, it appears that the progressive loss of the residual pancreatic function and store following STZ is delayed or prevented by vanadium treatment. In this respect, the degree of β-cell protection by vanadium may also depend on a critical number of β cells that initially survive STZ toxicity, and hence not all diabetic animals treated with vanadium may demonstrate sustained euglycemia following withdrawal from vanadium treatment. In support of this notion, in rats administered high-dose STZ (75 mg/kg), hyperglycemia was found to recur in all animals after vanadium treatment was withdrawn.[49] In a similar manner, insulin treatment could not induce a reversal of the diabetic state following high-dose STZ (≥60 mg/kg[44]).

In conclusion, short-term vanadium treatment eliminated hyperglycemia in a significant proportion of STZ rats after treatment was stopped. This phenomenon was linked to a modest, albeit significant, improvement in pancreatic insulin content, and was associated with an increased number of granulated β cells per islet. Since vanadium pretreatment did not prevent STZ-induced β-cytotoxicity, it appears that the vanadium-induced amelioration of the diabetic state may be secondary to the preservation of a functional portion of pancreatic β cells that initially survive the STZ-induced β-cytotoxicity. Furthermore, these results demonstrate that apparently minor changes in the islet insulin store in a model of reduced β-cell mass can have profound consequences for glucose homeostasis in the long term, and may have important implications for interventions that have "limited" effects on β cells.

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