EXPERIMENTAL STUDY

Distinct glucose lowering and beta cell protective effects of vanadium and food restriction in streptozotocin-diabetes

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Abstract

Vanadium is an oral insulin-mimetic agent that diminishes hyperglycemia, improves beta-cell insulin store and secretory function, and can reverse the diabetic state chronically after withdrawal from treatment. As food restriction has been reported to enhance insulin sensitivity and reduce insulin demand, we assessed the contribution of a reduced food intake to the glucose lowering and beta-cell protective effects of vanadium. Streptozotocin (STZ)-diabetic rats were untreated (D) or administered vanadyl sulfate in the drinking water (DT) at one week prior to and for 5 weeks following the administration of STZ. An additional group was pair-fed (DP) with an equal amount of food as that consumed by the DT group. Shortly after the induction of diabetes, hyperglycemic D rats demonstrated a significant rise in plasma insulin to levels that initially exceeded that of the controls. This was followed by a steady reduction over several weeks, suggesting a gradual depletion of functional beta-cells. Both vanadium treatment and pair-feeding abolished the insulin hypersecretory response following STZ administration. Glucose lowering was enhanced in DT animals when administered higher concentrations of vanadium, despite no further reduction in food intake, and all DT animals (10/10) were normoglycemic by 5 weeks. Mean pancreatic insulin content in DT rats was improved fourfold and was associated with a greater number of granulated beta-cells. Conversely, food restriction only modestly improved glycemia and the pancreatic insulin store and, unlike DT, DP rats remained highly glucose-intolerant. At 5 weeks of diabetes, fed circulating glucose and insulin levels were strongly correlated ($P=0.0002$) in the D and DP groups, supporting the notion that glucose lowering with food restriction is dependent on improved plasma insulin levels. A separate correlation was observed in DT animals within a lower range of plasma insulin, suggesting that vanadium, unlike food restriction, reduced plasma glucose by enhancing insulin sensitivity. Thus, vanadium preserves beta-cells in STZ-diabetes at least partially by abolishing the insulin hypersecretory response and the eventual exhaustion of residual insulin stores following a moderate dose of STZ. This property of vanadium would appear to be useful in the treatment of prediabetic and newly diagnosed insulin-dependent diabetes mellitus.

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Introduction

Vanadium is a ubiquitous trace element demonstrated to have glucose-lowering properties and improve pancreatic insulin store and secretory function in streptozotocin (STZ)-diabetic rats (1–3). Chronic vanadium treatment also reverses the diabetic state for up to 20 weeks after removal from treatment, a phenomenon associated with increased plasma insulin levels and an enhanced beta-cell mass (4, 5). Consequently, we demonstrated that short-term treatment with vanadium prior to and for a short period (2 weeks) following the induction of STZ-diabetes eliminated hyperglycemia after withdrawal from treatment (6). Chronic amelioration of the diabetic state in these animals was related to

a small but significant increase in the pancreatic insulin store. We had hypothesized that perhaps the removal of hyperglycemia with vanadium treatment had prevented the functional and structural exhaustion of existing beta-cells.

An auxiliary effect of vanadium treatment in both control and diabetic rats is a significant reduction in food intake, suggested to be due to a specific anorexiogenic stimulus in the central nervous system (7). Previous studies have reported a similar reduction of hyperglycemia by vanadium treatment and pair-feeding in STZ-diabetic animals (8, 9), hence complicating the interpretation of insulin-like properties of vanadium *in vivo*. In addition, like vanadium, food restriction in STZ-diabetic rats can reduce hyperglycemia and

increase insulin sensitivity as measured by hyperinsulinemic clamp (10). Chronic food restriction has also been demonstrated to enhance insulin-mediated glucose uptake and reduce insulin demand in control rats (11–13). Furthermore, a significant improvement in insulin sensitivity and delayed progression of loss of endogenous insulin was observed in adult-onset insulin-dependent diabetes mellitus (IDDM) patients on a normal protein as opposed to a high protein diet (14). Thus, it is plausible that a lowered food intake *per se* may contribute significantly to both the glucose lowering and beta-cell protective effects of vanadium. In the present study, we compared the effectiveness of chronic vanadium treatment or food restriction on the overall amelioration of the diabetic state. Vanadium treatment or pair-feeding was initiated 1 week prior to the STZ injection in order to acclimatize the animals and to stabilize the level of food intake during and immediately following the administration of STZ.

Materials and methods

Treatment and maintenance of animals

Male Wistar rats (170–200 g, 7–8 weeks old) were obtained from Charles River (St Constante, Quebec, Canada). The animals were maintained on standard laboratory chow and housed in individual wire-bottom cages. The rats were divided into the following groups: nondiabetic control (C, $n=4$), and untreated (D, $n=10$), vanadyl-treated (DT, $n=10$), and pair-fed (DP, $n=10$) diabetic groups. Vanadyl sulfate (0.75–1.00 mg/ml, Fisher Scientific Co., Fair Lawn, NJ, USA) was provided in the drinking water to the DT group for 7 days prior to STZ injection as described previously (5). In addition, a pair-fed group was provided with the same amount of food as that consumed by the DT group. After 7 days of vanadium treatment or pair-feeding, STZ (55 mg/kg *i.v.*, Sigma Chemical Co., St Louis, MO, USA) was administered to the diabetic rats, while the control group received vehicle (NaCl, 154 mmol/l, pH 7.2). Continuation of vanadium treatment (1 mg/ml) for 1 week after STZ resulted in normoglycemia in 1/10 DT rats. In an effort to increase the normoglycemic response in the DT group, the concentration of vanadium was gradually raised by increments of 0.25 mg/ml in all DT animals, to a maximum concentration of 1.75 mg/ml by 5 weeks. Plasma glucose, insulin, body weight, and daily food and fluid intake were monitored throughout the study. Food intake in DT animals was measured twice daily at 0900 h and 1800 h to account for nocturnal and daytime food consumption respectively. The average amount of food consumed was then given to the DP animals at 1800 h and 0900 h to mimic the feeding pattern of DT animals. To measure food intake accurately in the DT and DP groups, weighing trays were placed underneath the feed troughs to account for food that had not been consumed.

Animals were killed at 5 weeks post-STZ, and plasma obtained for measuring glucose and triglycerides (Boehringer Mannheim, Laval, Quebec, Canada). Insulin was measured by a radioimmunoassay (RIA) using rat insulin standards (Novo Research Institute, Copenhagen, Denmark), with inter- and intra-assay coefficients of variation of <10% and a sensitivity to 7 μ U/ml (15).

Oral glucose tolerance test (OGTT)

At 5 weeks after STZ, all rats were fasted for 16 h overnight (1800–0900 h). Pair-fed rats were not given their second daily ration of food at 1800 h, hence the last time food had been provided to DP animals prior to the OGTT was at 0900 h the previous day. The fasted animals were administered glucose (1 g/kg of a 40% glucose solution) by oral gavage. Blood samples (350 l) were collected from the nicked tail prior to (time 0) and at 10, 20, 30 and 60 min following the glucose dose. Plasma was stored frozen at -70°C for measurement of insulin and glucose levels.

Pancreatic insulin

At time of death, pancreata were dissected, cleared of lymph nodes and fat, blotted and weighed. The pancreas was immediately homogenized in 5 ml cold 2 M acetic acid for 5 s, and boiled for 10 min. The extract was centrifuged at 15 000 r.p.m. for 10 min, and the resulting supernatant was frozen at -70°C until further analysis of insulin.

Islet histology

A portion of pancreas was fixed in 2% formalin for 1–2 days, dried and embedded in paraffin. Sections were stained for granulated beta-cells by the modified aldehyde fuchsin method as previously described (16) and examined by light microscopy.

Vanadium levels

At termination, kidney, liver, muscle, bone, pancreas and plasma samples were analyzed for vanadium levels via atomic absorption using a method previously described (17).

Statistical analysis

One-way analysis of variance (ANOVA) was used followed by the Newman–Keuls test. Data are expressed as means $\pm$ S.E.M. $P<0.05$ was considered significant.

Results

Glycemia and food intake

Diabetic rats demonstrated a progressive rise in food intake that reached twofold that of control rats by 5

weeks post-STZ (Fig. 1A). Prior to STZ, vanadium treatment (1 mg/ml) reduced food intake to 80% of controls, and this remained unchanged both after the STZ injection and after the vanadium concentration was raised to a maximum of 1.75 mg/ml. However, at higher vanadium concentrations, animals that were previously unresponsive became normoglycemic, such that all (10/10) DT rats had normal plasma glucose by week 5 (Fig. 1B). Approximately 1/3 of the total daily food intake in DT animals was consumed between 0900–1800 h and ~2/3 between 1800–0900 h, and hence pair-fed animals were provided with similar amounts of food during these time intervals. However, unlike the DT animals, pair-fed rats would rapidly consume their entire food ration within the first hour, and hence were fasted for extended periods on a daily basis – from 1100–1800 h (7 h) and from 1900–0900 h (14 h). As blood was routinely collected at 0900–1000 h, plasma glucose and insulin were effectively 14-h fasted values in the pair-fed animals that, nevertheless, remained hyperglycemic. Thus, although mean plasma glucose in the DP group was significantly reduced at week 5, none of these animals developed normoglycemia.

Prior to STZ, vanadium treatment and food restriction for one week reduced body weight gain to

Figure 1 Effects of vanadium treatment and pair-feeding on food intake and plasma glucose levels in STZ-diabetic rats over 6 weeks. Food intake (A) and plasma glucose (B) of control (C, ○), and untreated (D, □), vanadium-treated (DT, ●) and pair-fed (DP, ■) diabetic groups over 6 weeks are shown. The numbers of normoglycemic animals in the DT group are shown in parentheses. * $P<0.05$ vs C, + $P<0.05$ vs D. Vanadium (V) concentrations are indicated above the X-axis.

the same extent (data not shown). However, at 5 weeks after the induction of diabetes, pair-fed animals had a more pronounced reduction in weight gain relative to the DT group (body weight: DP, 246 ± 8 g vs DT, 284 ± 9 g, $P<0.05$). Plasma triglyceride levels were also significantly reduced in the DP animals (0.44 ± 0.05 mmol/l) relative to all other groups (C, 1.20 ± 0.19 ; D, 1.37 ± 0.32 ; DT, 1.25 ± 0.15 mmol/l).

Oral glucose tolerance test

After 16 h of fasting, mean glycemia in DT animals was not significantly different from controls (Fig. 2A). In contrast, and despite having been fasted for >22 h, DP rats had relatively high basal glucose (~12 mmol/l) that was not different from the untreated diabetic group. Vanadium-treated rats showed an improved glucose response, with a return to control levels by 60 min, whereas pair-fed rats continued to demonstrate marked glucose intolerance, similar to the D group. The insulin secretory response in all diabetic groups was markedly

Figure 2 Effect of vanadium treatment and pair-feeding on oral glucose tolerance at 5 weeks. Plasma glucose (A) and insulin (B) responses to 1 g/kg oral glucose dose in overnight-fasted rats in control (C, ○), and untreated (D, □), vanadium-treated (DT, ●) and pair-fed (DP, ■) diabetic groups over 60 min are shown. Inset shows insulin release in enhanced Y-axis scale. * $P<0.05$ vs basal.

reduced relative to control (Fig. 2B). Although no changes over basal insulin were observed in the DP and D groups, DT rats demonstrated a significant peak in insulin (over basal) in response to glucose that returned to basal as glucose levels dropped (Fig. 2B, inset).

Plasma insulin

Initial plasma insulin levels were similar between control and diabetic animals taken prior to the STZ injection (Fig. 3A). In control rats, plasma insulin was found to increase steadily over 5 weeks, paralleling weight gain (~200 g) over the same time period. Interestingly, plasma insulin in D animals gradually rose to levels that exceeded that of control animals for 2 weeks following STZ, but subsequently dropped to 50% of controls by 5 weeks. Prior to the induction of diabetes, plasma insulin was significantly reduced by one week of vanadium treatment but not by pair-feeding. However, both vanadium treatment and pair-feeding abolished the temporal rise in plasma insulin following STZ, and vanadium treatment lowered it to a greater extent than pair-feeding on day 3. Plasma insulin and glucose values (averaged over 4 readings during the final 2 weeks of the study) were strongly correlated ($r = -0.74$, $P = 0.0002$) in the D and DP groups, but this correlation excluded the DT animals which had both low plasma glucose and insulin levels (Fig. 3B). Nevertheless, the DT group demonstrated a distinct correlation between plasma glucose and insulin levels over a narrower range ($r = -0.68$, $P = 0.03$) (enhanced Y-axis scale, inset). No similar correlation was observed in the control group.

Variable response to vanadium treatment

As we reported previously (4), some diabetic animals became normoglycemic at relatively low vanadium concentrations (DT-LC, 1.00–1.25 mg/ml, $n = 4$) whereas others required higher levels (DT-HC, 1.50–1.75 mg/ml, $n = 6$) to achieve normoglycemia (Fig. 4A). Although plasma glucose levels in the DT-HC subgroup were significantly higher than the DT-LC subgroup for 4 weeks after STZ, plasma insulin levels were equally low over the entire treatment duration in both DT subgroups (Fig. 4B). Although the same concentrations were provided to all DT animals regardless of response, the calculated dose per body weight was significantly greater in the DT-HC subgroup until week 4 because of the higher fluid intake in these animals (Fig. 4C). Fluid intake subsequently dropped as the DT-HC animals gradually responded to treatment. At time of death, vanadium levels (nmol/ml or nmol/g) in DT animals were: plasma, 7.8 ± 1.0 ; liver, 30.6 ± 3.7 ; muscle, 4.5 ± 0.2 ; pancreas, 8.0 ± 1.0 ; bone, 415.0 ± 40.4 ; kidney, 129.4 ± 6.9 . These levels are similar to previously reported values in diabetic rats after one year of vanadium treatment (18). Vanadium levels were not

Figure 3 Effect of vanadium treatment and pair-feeding on plasma insulin. Plasma insulin levels (A) and correlation between plasma glucose and insulin levels averaged over 4 readings (final 2 weeks) prior to death (B) in control (C, ○), and untreated (D, □), vanadium-treated (DT, ●) and pair-fed (DP, ■) diabetic groups. * $P < 0.05$ vs C, + $P < 0.05$ vs DP. Inset shows correlation among DT animals in enhanced Y-axis scale.

statistically different between the DT-HC and DT-LC subgroups at 5 weeks.

Pancreatic insulin content and islet histology

Total pancreatic insulin content was dramatically reduced to <15% of controls (1.6 ± 0.1 mU/g) in all diabetic rats at 5 weeks after STZ. However, relative to D,

Figure 4 Plasma glucose, insulin and self-administered vanadium dose in DT subgroups. Fed plasma glucose (A), insulin (B) and vanadium-treated diabetic animals showing subgroups normalized at low (DT-LC, 1.00–1.25 mg/ml, ●, $n=4$) and high (DT-HC, 1.50–1.75 mg/ml, ○, $n=6$) concentrations of vanadyl sulfate in the drinking water (indicated above the X-axis). * $P<0.05$ vs DT-LC.

the residual insulin store was higher in DT and DP groups by ~4- and 2-fold respectively (Fig. 5A). Within the DT group, those animals that had required low concentrations of vanadium to achieve normoglycemia (DT-LC subgroup) had a residual insulin content that was consistently greater than the DT-HC subgroup that had required higher concentrations of vanadium to achieve normoglycemia. There was a strong correlation between residual insulin store and fed glycemia in the D group ($r = -0.93$, $P = 0.0003$) (Fig. 5B), which was similar to our previous observations in untreated diabetic rats (6). DP animals also demonstrated a strong correlation ($r = -0.88$, $P = 0.0007$) that was distinct from D, whereas no correlation was found in DT animals. The level of pancreatic insulin store found within the DT-LC subgroup (>150 mU/g) is sufficient for the maintenance of normoglycemia in the absence of vanadium (6). Aldehyde-fuchsin staining revealed that

the beta-cells in islets of control animals were well granulated (Fig. 6A), whereas those from the untreated (Fig. 6B) and pair-fed (Fig. 6D) diabetic groups were degranulated. The vanadium-treated animals consistently showed a greater degree of beta-cell granulation relative to the other diabetic groups (Fig. 6C).

Discussion

Pharmacological interventions that effectively stabilize a functional beta-cell mass at the onset of Type 1 diabetes have been shown to induce a chronic amelioration of the diabetic state (19). In rats, pretreatment with exogenous insulin for 5 days offset the diabetogenic action of STZ by preserving 30% of the pancreatic insulin content, an effect attributed to a reduced activity of beta-cells at the time of STZ administration (20). In contrast, increased metabolic

Figure 5 Pancreatic insulin content. (A) Pancreatic insulin content and (B) correlation with plasma glucose levels in the various diabetic groups: untreated ($\square$), treated (DT) rats normalized following low (DT-LC, 1.00–1.25 mg/ml, $\bullet$) and high (DT-HC, 1.50–1.75 mg/ml, $\circ$) vanadyl concentrations and pair-fed ($\blacksquare$) groups. Dashed line represents correlation in the untreated D group.

activity by incubating at high glucose concentration enhanced susceptibility of beta-cells to STZ (21). We have previously demonstrated that vanadium treatment can induce a chronic reversal of the diabetic state long after treatment is ceased, an effect linked to improvements in both pancreatic insulin store and secretory function (4, 5). Consequently, we found that, whereas 1-week vanadium pretreatment did not prevent the onset of diabetes, continued treatment for 2 weeks after STZ effectively reversed the diabetic state in 50% of the animals (6). Thus, the protection of beta-cells by vanadium cannot be due to its effects at the time of STZ administration, but more likely depends on events following the induction of diabetes. In this respect, short-term insulin treatment following STZ in neonatal rats was also found to enhance the spontaneous remission of diabetes (22). In humans, intensive insulin treatment for 2 weeks following recent-onset diabetes results in the preservation of residual beta cell function and insulin content for at least one year (23, 24). This

effect was attributed to a reduced insulin demand, as direct inhibition of insulin secretion by diazoxide also preserved beta cells in Type 1 diabetic patients (25), and reduced the incidence of diabetes in spontaneously diabetic BB (26) and 90% pancreatectomized rats (27).

In this study, untreated diabetic animals experienced a surge in circulating insulin levels for 2 weeks following STZ, indicating that residual, viable beta cells had maintained some insulin secretory function. A subsequent decline in insulin levels over several weeks suggests a gradual depletion of the residual insulin store that was <5% at 5 weeks. These observations are consistent with a study by Junod *et al.* who demonstrated that, following a moderate dose of STZ (55 mg/kg), the residual insulin reserve was further depleted to <5% of control by 4 weeks (28). The high circulating insulin following STZ may have resulted from a sustained compensatory response of the remaining beta cells to the prevailing hyperglycemia. On the other hand, Leahy *et al.* (29) reported that 90% pancreatectomized rats had beta-cells that were intrinsically more sensitive to glucose and secreted insulin at 90% of their capacity, leading to a loss of glucose-induced insulin secretion. Both vanadium treatment and food restriction effectively abolished the temporal insulin hypersecretory phase following STZ, and resulted in significantly improved insulin stores. Hence, it appears that vanadium and, to a lesser extent, food restriction may have preserved the residual beta-cell mass by preventing its gradual exhaustion.

Vanadium has been reported to reduce plasma insulin levels in animals that exhibit hyperinsulinemia such as obese *fa/fa* (30), spontaneously hypertensive (31) and fructose-hypertensive (32) rats. Notably, administration of vanadate to hyperinsulinemic *ob/ob* mice reduced plasma insulin levels, which led to a fourfold improvement in insulin stores by 7 weeks (33). In this study, the apparent inhibition of insulin secretion by vanadium was not secondary to its glucose lowering effect as both DT-LC and DT-HC animals experienced a similar reduction in circulating insulin despite markedly different levels of glycemia. This suggests that vanadium can exert a direct inhibitory effect on insulin secretion in pancreatic beta-cells. High concentrations of vanadium (0.1–1 mmol/l) *in vitro* have been shown to stimulate insulin secretion from rat islets (34, 35). However, inhibition of glucose-stimulated insulin release was demonstrated at vanadium levels (<10 μ mol/l) similar to those detected in plasma and pancreas both in this and in previous studies (36). Aside from a sustained inhibitory effect on insulin release, it is also possible that normalization of glucose levels *per se* (for instance, by administering phlorizin) could reduce functional demand and result in the partial protection of beta-cells. Interestingly, several elements that were found to have glucose-lowering properties in STZ-diabetic rats, such as molybdenum, selenium and tungsten, were similarly shown to

Figure 6 Islet histology. Photomicrographs of islets stained for beta-cells in control (A), and in diabetic untreated (B), vanadium-treated (C) and pair-fed (D) groups. Original magnification $\times 500$.

induce slight to significant increases in insulin store or glucose-stimulated insulin secretion (37–39). Furthermore, tungsten increased the incorporation of [^3H]thymidine to islet cell lines, suggesting a direct trophic effect (39). Whether vanadium or the other elements have direct positive effects on beta-cell function, either by enhancing beta-cell regeneration or by increasing insulin gene expression, requires further study.

The degree of beta-cell protection afforded by vanadium, to the extent that some treated animals experience a chronic reversal of the diabetic state, may depend on the relative number of beta cells that initially survived destruction by STZ. Thus, more severely diabetic animals that had required higher concentrations of vanadium to achieve normoglycemia were also more likely to revert to hyperglycemia once treatment was withdrawn, whereas those requiring relatively lower doses remained normoglycemic for 5 months after removal of vanadium (4). In this regard, insulin treatment for 7 days following the administration of STZ normalized islet morphology and insulin secretory response and reversed the diabetic state for 3 months following moderate (50 mg/kg) but not high (60–70 mg/kg) doses of STZ (22). Similarly, following a high STZ dose (75 mg/kg), hyperglycemia recurred in all animals after vanadium treatment was withdrawn (40). It is notable in this study that the more responsive DT-LC animals were also ones that maintained a critical level of pancreatic insulin store (150 mU/g) previously

found to support normoglycemia *per se* (6), and hence can be predicted to remain normal had treatment been withdrawn. Although food restriction similarly reduced plasma insulin levels throughout the entire study, the relative improvement in residual insulin store in DP animals was still less than that resulting from vanadium treatment. It should be noted that blood was collected at a time when pair-fed animals had been without available food for at least 14 h, and that plasma insulin levels could have been consistently higher during the time of feeding. Additionally, plasma insulin in the DT group was significantly lower than the pair-fed group at 3 days after STZ. As the residual insulin store in DP rats remained well below the critical level required to support normoglycemia *per se*, it is likely that the moderate lowering of plasma glucose observed in some pair-fed animals would have been reversed had normal (food available *ad libitum*) feeding resumed.

Recent studies indicate that food restriction has no beneficial effect on hepatic glucose metabolism (38) or cardiac dysfunction (41) in STZ-diabetic rats. Moreover, as vanadium was demonstrated to have acute (within minutes) glucose lowering effects (42), this would rule out the contribution of a reduced food intake to explain its hypoglycemic action. This study also confirms that the glucose lowering effects due to vanadium are separate from those of a reduced food intake (8, 9). First, an enhanced glucose lowering response with higher vanadium doses did not accompany a further

reduction in food intake. In addition, effects of a reduced food intake on glycemia were relatively modest compared with the dramatic reduction in response to vanadium treatment, and DP rats remained highly glucose-intolerant. Finally, unlike pair-fed and untreated diabetic rats that showed a strong correlation between glycemia and insulin (both plasma and pancreatic), normoglycemia induced by vanadium treatment did not necessitate an increased availability of insulin. Notably, 3 DT rats had pancreatic insulin content <60 mU/g, levels that did not support normoglycemia in either D or DP rats. This notion of a complementary action between vanadium and insulin is inherent in the observation that more severely diabetic animals, which had a lower residual pancreatic insulin content at 5 weeks, also required higher concentrations of vanadium to achieve normoglycemia. However, although the effectiveness of vanadium is initially dependent on the residual insulin stores, when administered at higher concentrations it can elicit normoglycemia even in animals with insulin stores as low as 2.5% of control. As beta-cell function in STZ-diabetes is very closely correlated with pancreatic insulin content and intercepts at zero (43), it would mean that vanadium could continue to exert its glucose lowering effects even when pancreatic secretory function is severely diminished.

A possible explanation for the dramatic reduction in glucose levels by food restriction in previous studies (8, 9) was that sporadic feeding produced prolonged periods of starvation. Despite feeding these animals at two intervals per day, plasma glucose levels actually represented 14-h fasted values as DP rats consumed all their food within 1 h. In comparison, previous studies using a once-daily feeding schedule would probably have measured glucose levels following an even longer period of fasting. Indeed, in an earlier report by the same group, food-restricted diabetic rats rapidly consumed the available food (44). Thus, as bleeding was routinely carried out at 0900 h, pair-fed diabetic rats were probably fasted for at least 22 h after having been fed at 0900 h the previous day (9). Thus, the dramatic reduction in glycemia induced by food restriction in diabetic rats can arise from poorly controlled feeding which, in conjunction with the hyperphagic behavior of diabetic rats, results in prolonged fasting.

In summary, vanadium preserves beta-cells in STZ-diabetes at least in part by preventing the beta-cell hypersecretory response and exhaustion of residual insulin stores following a moderate dose of STZ. In this way, short-term treatment with vanadium can reverse the diabetic state by preserving a small, albeit critical, amount of insulin store that adequately supports normoglycemia even after long-term withdrawal from treatment. This inhibitory effect of vanadium on insulin secretion is not dependent on a reduction of plasma glucose levels, and might be a direct effect on beta-cells. In addition, we show that the glucose-lowering effect of

vanadium is not dependent on an increased availability of plasma or pancreatic insulin as observed in pair-fed animals, thus ruling out the notion that a reduced food intake contributes significantly to the amelioration of diabetes by vanadium. Hence, the combined effects of vanadium in eliciting normoglycemia at remarkably low insulin reserves and in preserving residual insulin stores suggests that it may prevent the progressive deterioration of beta-cells in prediabetic and newly diagnosed IDDM.

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References

- 1 Blondel O, Bailbe D & Portha B. *In vivo* insulin resistance in streptozotocin-diabetic rats – evidence for reversal following oral vanadate treatment. *Diabetologia* 1993 **32** 185–190.
- 2 Brichard SM, Okitolonda W & Henquin JC. Long term improvement of glucose homeostasis by vanadate treatment in diabetic rats. *Endocrinology* 1988 **123** 2048–2053.
- 3 Cam MC, Pederson RA, Brownsey RW & McNeill JH. Long-term effectiveness of oral vanadyl sulphate in streptozotocin-diabetic rats. *Diabetologia* 1993 **36** 218–224.
- 4 Cam MC, Faun J & McNeill JH. Concentration-dependent glucose-lowering effects of oral vanadyl are maintained following treatment withdrawal in streptozotocin-diabetic rats. *Metabolism* 1995 **44** 332–339.
- 5 Pederson RA, Ramanadham S, Buchan AMJ & McNeill JH. Long-term effects of vanadyl treatment on streptozotocin-induced diabetes in rats. *Diabetes* 1989 **28** 1390–1395.
- 6 Cam MC, Li WM & McNeill JH. Partial preservation of pancreatic β -cells by vanadium: evidence for a mechanism of chronic amelioration of diabetes. *Metabolism* 1997 **46** 769–778.
- 7 Meyerovitch J, Shechter Y & Amir S. Vanadate stimulates *in vivo* glucose uptake in brain and arrests food intake and body weight gain in rats. *Physiology and Behavior* 1989 **45** 1113–1116.
- 8 Malabu UH, Dryden S, McCarthy D, Kilpatrick A & Williams G. Effects of chronic vanadate administration in the STZ-induced diabetic rat. The anti-hyperglycemic action of vanadate is attributable entirely to its suppression of feeding. *Diabetes* 1994 **43** 9–15.
- 9 Domingo JL, Gomez M, Sanchez DJ, Llobet JM, Corbella J & Keen CL. Normalization of hyperglycemia by vanadate or vanadyl treatment in diabetic rats: pharmacological and toxicological aspects. *Trace Elements in Medicine* 1994 **11** 16–22.
- 10 Cameron-Smith D, Collier GR & O'Dea K. Reduction in hyperglycemia by mild food restriction in streptozotocin-induced diabetic rats improves insulin sensitivity. *Hormone and Metabolic Research* 1994 **26** 316–321.
- 11 Chu KU, Ishizuka J, Poston GJ, Townsend CM, Greeley GH, Yu BP *et al.* Change in endocrine pancreatic function in short-term diet restriction. *Nutrition* 1991 **7** 425–429.
- 12 Escriva F, Rodriguez C, Cacho J, Alvarez C, Portha B & Pascual-Leone AM. Glucose utilization and insulin action in adult rats submitted to prolonged food restriction. *American Journal of Physiology* 1992 **263** E1–E7.
- 13 Picarelblanchot F, Alvarez C, Bailbe D, Pascualleone AM & Portha B. Changes in insulin action and insulin secretion in the rat after

- dietary restriction early in life – influence of food restriction versus low-protein food restriction. *Metabolism* 1995 **44** 1519–1526.
- 14 Linn T, Geyer R, Prassek S & Laube H. Effect of dietary protein intake on insulin secretion and glucose metabolism in insulin-dependent diabetes mellitus. *Journal of Clinical Endocrinology and Metabolism* 1996 **81** 3938–3943.
 - 15 Cam MC & McNeill JH. A sensitive radioimmunoassay optimized for reproducible measurement of rat plasma insulin. *Journal of Pharmacological and Toxicological Methods* 1996 **35** 111–119.
 - 16 Mowry RW, Longley GB & Emmel VM. Only aldehyde-fuchsin made from pararosanilin stains pancreatic β -cell granules and elastic fibers in unoxidized microsection: problems caused by mislabeling of certain basic fuchsin. *Stain Technology* 1980 **55** 91–103.
 - 17 Mongold JJ, Cros GH, Vian L, Tep A, Ramanadham S, Siou G *et al.* Toxicological aspects of vanadyl sulphate on diabetic rats: effects on vanadium levels and pancreatic β -cell morphology. *Pharmacology and Toxicology* 1990 **67** 192–198.
 - 18 Dai S, Thompson KH, Vera E & McNeill JH. Toxicity studies on one-year treatment of non-diabetic and streptozotocin-diabetic rats with vanadyl sulphate. *Pharmacology and Toxicology* 1994 **75** 265–273.
 - 19 Elliott JE. New approaches to preventing and treating Type 1 diabetes: discovering a method to preserve β cell mass after diagnosis should remain a key research focus. *Canadian Journal of Diabetes Care* 1998 **22** S24–S30.
 - 20 Thulesen J, Orskov C, Holst JJ, Seier S & Poulsen S. Short term insulin treatment prevents the diabetogenic action of streptozotocin in rats. *Endocrinology* 1997 **138** 62–68.
 - 21 Eizirik DL, Strandell E & Sandler S. Culture of mouse pancreatic islets in different glucose concentrations modifies β cell sensitivity to streptozotocin. *Diabetologia* 1988 **31** 168–174.
 - 22 Ar'Rajab A & Ahren B. Long-term diabetogenic effect of streptozotocin in rats. *Pancreas* 1993 **8** 51–57.
 - 23 Schnell O, Eisfelder B, Standl E & Ziegler AG. High-dose intravenous insulin infusion versus intensive insulin treatment in newly diagnosed IDDM. *Diabetes* 1997 **46** 1607–1611.
 - 24 Shah SC, Malone JI & Simpson NE. A randomized trial of intensive insulin therapy in newly diagnosed insulin-dependent diabetes mellitus. *New England Journal of Medicine* 1989 **320** 550–554.
 - 25 Bjork E, Berne C, Kampe O, Wibell L, Oskarsson P & Karlsson FA. Diazoxide treatment at onset preserves residual insulin secretion in adults with autoimmune diabetes. *Diabetes* 1996 **45** 1427–1431.
 - 26 Vlahos WD, Seemayer TA & Yale JF. Diabetes prevention in BB rats by inhibition of endogenous insulin secretion. *Metabolism* 1991 **40** 825–829.
 - 27 Leahy JL, Bumbalo LM & Chen C. Diazoxide causes recovery of β -cell glucose responsiveness in 90% pancreatectomized diabetic rats. *Diabetes* 1994 **43** 173–179.
 - 28 Junod A, Lambert AE, Stauffacher W & Renold AE. Diabetogenic action of streptozotocin: relationship of dose to metabolic response. *Journal of Clinical Investigation* 1969 **48** 2129–2139.
 - 29 Leahy JL, Bumbalo LM & Chen C. Beta-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. *Diabetologia* 1993 **36** 1238–1244.
 - 30 Brichard SM, Pottier AM & Henquin JC. Long-term improvement of glucose homeostasis by vanadate in obese hyperinsulinemic *fa/fa* rats. *Endocrinology* 1989 **124** 2510–2516.
 - 31 Bhanot S, Bryer-Ash M, Cheung A & McNeill JH. Bis(maltolato)oxovanadium(IV) attenuates hyperinsulinemia and hypertension in spontaneously hypertensive rats. *Diabetes* 1994 **43** 857–861.
 - 32 Bhanot S, McNeill JH & Bryer-Ash M. Vanadyl sulfate prevents fructose-induced hyperinsulinemia and hypertension in rats. *Hypertension* 1994 **23** 308–312.
 - 33 Brichard SM, Bailey CJ & Henquin JC. Marked improvement of glucose homeostasis in diabetic ob/ob mice given oral vanadate. *Diabetes* 1990 **39** 1326–1332.
 - 34 Fagin JA, Ikejiri K & Levin SR. Insulinotropic effects of vanadate. *Diabetes* 1987 **36** 1448–1452.
 - 35 Zhang A, Gao ZY, Gilon P, Nenquin M, Drews G & Henquin JC. Vanadate stimulation of insulin release in normal mouse islets. *Journal of Biological Chemistry* 1991 **266** 21649–21656.
 - 36 Voss C, Herrmann I, Hartmann K & Zuhlke H. *In vitro* effect of vanadate on content, secretion and biosynthesis of insulin in isolated islets of normal Wistar rats. *Experimental and Clinical Endocrinology* 1992 **99** 159–163.
 - 37 Ozcelikay AT, Becker DJ, Ongemba LN, Pottier AM, Henquin JC & Brichard SM. Improvement of glucose and lipid metabolism in diabetic rats treated with molybdate. *American Journal of Physiology* 1996 **270** E344–E352.
 - 38 Becker DJ, Reul B, Ozcelikay AT, Buchet JP, Henquin JC & Brichard SM. Oral selenate improves glucose homeostasis and partly reverses abnormal expression of liver glycolytic and gluconeogenic enzymes in diabetic rats. *Diabetologia* 1996 **39** 3–11.
 - 39 Barbera A, Fernandez-Alvarez J, Truc A, Gomis R & Guinovart JJ. Effects of tungstate in neonatally streptozotocin-induced diabetic rats: mechanism leading to normalization of glycemia. *Diabetologia* 1997 **40** 143–149.
 - 40 Bendayan M & Gringas D. Effect of vanadate administration on blood glucose and insulin levels as well as on the exocrine pancreatic function in streptozotocin-diabetic rats. *Diabetologia* 1989 **32** 561–567.
 - 41 Yuen VG, Orvig C & McNeill JH. Effects of bis(maltolato)oxovanadium(IV) are distinct from food restriction in STZ-diabetic rats. *American Journal of Physiology* 1997 **272** E30–E35.
 - 42 Yuen VG, Orvig C & McNeill JH. Comparison of the glucose-lowering properties of vanadyl sulfate and bis(maltolato)oxovanadium(IV) following acute and chronic administration. *Canadian Journal of Physiology and Pharmacology* 1994 **73** 55–64.
 - 43 McCulloch DK, Koerker DJ, Kahn SE, Bonner-Weir S & Palmer JP. Correlations of *in vivo* beta-cell function tests with beta-cell mass and pancreatic insulin content in streptozotocin-administered baboons. *Diabetes* 1991 **40** 673–679.
 - 44 McKibbin PE, McCarthy HD, Shaw P & Williams G. Insulin deficiency is a specific stimulus to hypothalamic neuropeptide Y: a comparison of the effects of insulin replacement and food restriction in streptozotocin-diabetic rats. *Peptides* 1992 **13** 721–727.

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