

Vanadate treatment rapidly improves glucose transport and activates 6-phosphofructo-1-kinase in diabetic rat intestine

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Summary The effect of oral vanadate on intestinal sodium-dependent glucose transport and 6-phosphofructo-1-kinase (EC 2.7.1.11) activity was examined in male Sprague-Dawley rats following a 30-day period of non-treated streptozotocin-induced diabetes. Non-treated diabetic rats were hyperglycaemic and demonstrated increased intestinal sodium-dependent glucose transport and Na,K-ATPase activity compared with controls. These increases were associated with a significant decrease in the total activity and activity ratios (activity at 0.5 mmol/l fructose 6-phosphate at pH 7.0/activity at pH 8.0) of intestinal 6-phosphofructo-1-kinase and decreased levels of fructose 2,6-bisphosphate. Supplementation of drinking water with vanadate (0.5 mg/ml) resulted in a rapid decline in blood glucose levels to a slightly hyperglycaemic level. Jejunal glucose transport and Na,K-ATPase activity were normalized after 48 h of vanadate treatment. In contrast, ileal glucose transport was significantly reduced 12 h following beginning vanadate treatment even though Na,K-ATPase activity did not normalize until 36 h later. K_m was significantly decreased in both jejunum and ileum by vanadate treatment indicating an increased affinity of the sodium-dependent intestinal glucose transporter for glucose. 6-phosphofructo-1-kinase total activity and susceptibility to ATP inhibition was completely re-

stored after 12 h of vanadate treatment. This increase was associated with a rise in fructose 2,6-bisphosphate levels. Fasting rats for 12 h had no effect on glucose transport or 6-phosphofructo-1-kinase activity, indicating the anorectic effect of vanadate was not responsible for changes in either parameter. In contrast, cycloheximide prevented both the rise in 6-phosphofructo-1-kinase activity and the rise in fructose 2,6-bisphosphate levels, and the subsequent reduction in glucose transport, indicating a requirement for protein synthesis. The removal of vanadate resulted in an immediate return to pre-treatment blood glucose levels. In contrast, intestinal glucose transport and 6-phosphofructo-1-kinase activity remained at treatment levels up until 72 h, indicating that oral vanadate treatment can have prolonged beneficial effects on intestinal function. In conclusion, the treatment of streptozotocin-induced diabetic rats with oral vanadate results in an activation of 6-phosphofructo-1-kinase coupled with a normalization of intestinal sodium-dependent glucose transport. Vanadate may thus have a beneficial effect on intestinal function and may prove useful as oral adjunctive diabetic therapy. [Diabetologia (1995) 38: 403–412]

Key words Glucose, intestine, vanadate, 6-phosphofructo-1-kinase.

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Abbreviations: PFK₁, 6-phosphofructo-1-kinase; SGLT, Na⁺-dependent glucose cotransporter; J_{max} , maximum transport rate; K_m , carrier affinity; STZ, streptozotocin; 3-O-MG, 3-O-methyl-D-glucopyranoside; PD, potential difference; GLUT2, glucose transporter 2; BBMV, brush border membrane vesicles
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Streptozotocin-induced (STZ) diabetes has been shown to result in an enhanced active intestinal absorption of glucose due to an increase in brush border Na-dependent glucose transporters (SGLT) [1] and basolateral membrane facilitated glucose carriers (GLUT2) [2, 3]. In addition, a reduction in enterocyte glucose utilization is seen in diabetes mellitus, primarily due to a decrease in the activity of 6-phosphofructo-1-kinase (PFK₁), [EC 2.7.1.11] which re-

sults in an indirect increase in glucose absorption [4]. The consequences of such an increase in intestinal glucose absorption may aggravate diabetes due to the enhanced rate of the postprandial rise in serum glucose.

Oral vanadate treatment has been demonstrated to have a serum glucose-lowering effect in the STZ diabetic rat model [5] and in genetic insulin-resistance animal models [6, 7]. Oral vanadate has also been shown to reduce Na-dependent glucose transport in the small intestine in normal rats and prevent the increase in transport from occurring in STZ-diabetic rats [8]. While there is evidence to suggest that oral vanadate is effective in restoring Na-dependent glucose transport to normal levels in the STZ-diabetic rat model [9], there have been no studies done to examine the effects of oral vanadate on intestinal PFK₁ activity and no studies to determine whether the jejunum and ileum response to vanadate is similar. Both a down-regulation of transport and an increase in glucose utilization, as a consequence of an increased PFK₁ activity, would be necessary to effectively decrease overall glucose absorption. In addition, it is not known if vanadate-induced intestinal effects are a consequence of decreasing blood glucose levels, or are due to a direct action of vanadate on intestinal enterocytes.

The purpose of this study was thus to examine the effects of oral vanadate treatment on Na-dependent glucose transport and PFK₁ activity in jejunum and ileum of the STZ-induced diabetic rat model, and to determine if these effects are linked with blood glucose levels.

Materials and methods

Materials. Streptozotocin was obtained from Upjohn (Don Mills, Ontario, Canada). Vanadate (V₂O₅) was purchased from Fisher Scientific (Edmonton, Alberta, Canada). D-[1-³H(N)]-glucose (15.5 Ci/mmol) was obtained from New England Nuclear (Boston, Mass., USA). The remainder of chemicals were reagent grade and were obtained from Sigma Chemical Company (St Louis, Mo., USA).

Animal model

Induction of diabetes. Diabetes was induced in male Sprague-Dawley rats (300–400 g) via a single i.p. dose of STZ (65 mg/kg). Blood glucose was measured 24 h after the STZ injection and at the time of study using an Ames reflectance photometer and glucose reagent strips (Ames Division, Miles Laboratory, Elkhart, Ind., USA). Only diabetic rats with hyperglycaemia of over 18 mmol/l were entered into the study.

Vanadate administration. Following a 30-day period of non-treated diabetes, vanadate (0.5 mg/ml) was added to the drinking water of rats for 7 consecutive days. In untreated diabetic animals, blood glucose levels were measured at 08.00–09.00 hours. Vanadate treatment was begun immediately thereafter

and 24-h-blood glucose levels were measured the following morning at 08.00–09.00 hours. For 12 h blood glucose levels, vanadate treatment was begun at 08.00–09.00 hours, and blood glucose levels and experiments performed the following morning at 08.00–09.00 hours. Fasted animals had their food removed for the same time periods (08.00–20.00 hours). All animals, except for the fasted group, were allowed access to the standard rat diet ad libitum, and housed in a light-cycled animal care facility.

Three groups were studied: (1) age-matched controls; (2) non-treated diabetic; and (3) vanadate-supplemented diabetic. In the vanadate-treated group, rats were studied at various time intervals after vanadate was added to the drinking water.

Intact mucosal sheet transport experiments

Segments of jejunum and ileum from which the outer muscle layer had been removed were mounted in Ussing Chambers and bathed on both sides with a bicarbonate-Ringer's solution containing 20 mmol/l fructose circulated with 5% CO₂/95% O₂ (pH 7.4) at 37°C. The spontaneous transepithelial potential difference (PD) and short circuit current (I_{sc}) was measured. Maximal transport capacity (J_{max}) and carrier affinity (K_m) were determined for 3-O-methyl-D-glucopyranoside (3-O-MG) (concentration range 0.5–60 mmol/l) by measurement of concentration-dependent short circuit current (I_{sc}) as previously described [10].

Enzyme analysis

Rats were killed with a pentobarbital overdose (240 mg/kg) and the jejunum and ileum removed and flushed with ice cold 0.9% NaCl. The mucosa was scraped off with a glass slide, weighed, and homogenized in a 1:10 volume of extraction buffer using a Polytron homogenizer.

Na,K-ATPase activity. Na,K-ATPase activity was assessed by incubating samples (50 µg protein/ml) in 25 mmol/l imidazole, 1 mol/l EGTA, and 0.6 mg deoxycholate/mg protein for 15 min at 37° to ensure the disruption of membranes and ligand access to all ouabain binding sites. To start the reaction, aliquots of the homogenate were added to buffer containing 50 mmol/l histidine, 130 mmol/l NaCl, 20 mmol/l KCl, 4 mmol/l MgCl, and 3 mmol/l ATP (pH 7.4). The reaction was allowed to proceed for 15 min at 37°C and then quenched by the addition of ice cold 8% perchloric acid. Samples were centrifuged, and inorganic phosphate levels were measured using the method of Radominski-Pyret et al. [11]. Samples to which protein was added after the reaction was terminated were used as blanks. Na,K-ATPase activity was calculated as the difference between inorganic phosphate released in the presence and absence of ouabain. Ouabain-sensitive ATP hydrolysis was in the range of 7% to 22% of total ATP hydrolysis, and this figure did not differ between the animal groups. Na,K-ATPase activity was based on the protein content of samples as measured using the Bradford technique [12].

6-phosphofructo-1-kinase activity. Total activity of the rate-limiting enzyme of glycolysis, PFK₁ (EC 2.7.1.11) was measured using the method described by Ling et al. [13]. Intestinal mucosa was removed and immediately frozen in liquid N₂ for measurement of PFK₁ and fructose 2,6-bisphosphate levels. To measure PFK₁, the frozen tissue was homogenized with 5 vol (volume/weight) of icecold extraction buffer solution

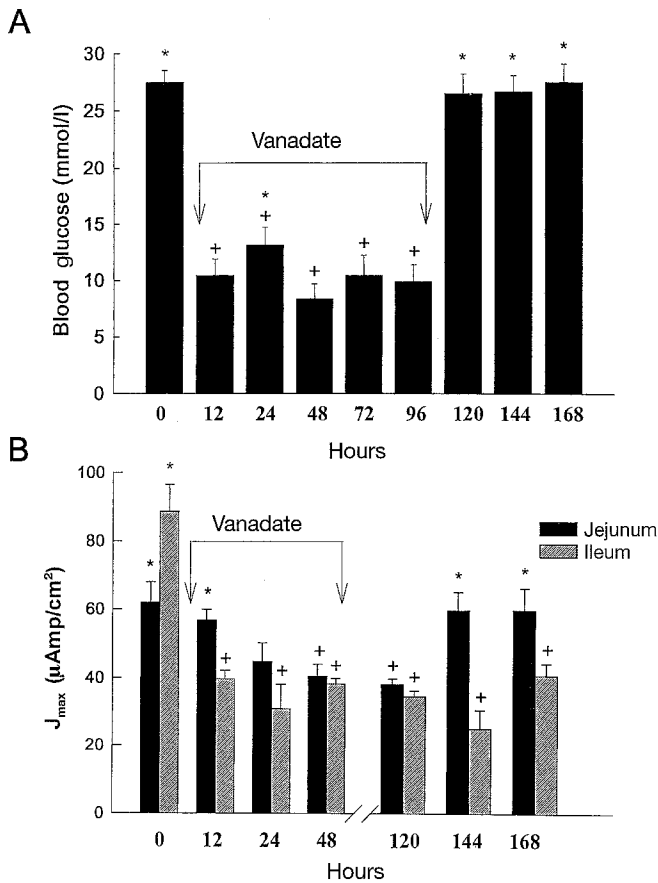


Fig. 1A, B. Blood glucose levels in diabetic rats following the initiation of vanadate treatment (time 0) and the removal of vanadate therapy (96 h later) (A). Vanadate treatment resulted in a rapid decline of blood glucose within 12 h to a slightly hyperglycaemic level whereby levels stabilized. Removal of vanadate treatment resulted in a return to pre-treatment levels within 24 h. * $p < 0.05$ relative to controls; + $p < 0.05$ relative to non-treated diabetics. Jejunal and ileal J_{max} for glucose transport measured in intact sheets of tissue in Ussing chambers as a response to vanadate treatment (B). Jejunal J_{max} was normalized after 48 h of vanadate treatment. Ileal J_{max} rapidly fell to near normal levels after 12 h. Jejunal J_{max} increased to pre-treatment levels 48 h following removal of vanadate. Ileal J_{max} began to increase to pre-treatment levels 72 h following removal of vanadate. * $p < 0.05$ relative to controls; + $p < 0.05$ relative to untreated diabetics. Each data point represents the mean \pm SEM of at least eight animals

(50 mmol/l Tris-HCl; 100 mmol/l $(\text{NH}_4)_2\text{SO}_4$; 30 mmol/l KF; 2 mmol/l DTT; 1 mmol/l EDTA; 1 mmol/l phenylmethanesulphonyl fluoride; and 0.5 mg/ml soybean trypsin inhibitor). Tissue homogenates were centrifuged for 30 min at 75 000 g at 4°C. Pellets were discarded and the supernatant used for assay. Activity of PFK₁ is expressed as the maximal activity at pH 8.0 where the enzyme is not subject to allosteric regulation [18]. The regulatory properties of PFK₁ are presented as the activity ratio ($v_{0.5}/V$) where $v_{0.5}$ is the suboptimal activity at pH 7.0 in the presence of 2.5 mmol/l ATP and 0.5 mmol/l fructose 6-phosphate under the conditions defined by Hussey et al. [14] and V is the total activity at pH 8.0.

Fructose 2,6-bisphosphate levels. Fructose 2,6-bisphosphate was measured in rat jejunal and ileal mucosa using the method of Van Schaftingen et al. [15] with stimulation to pyrophos-

phate-dependent phosphofructokinase (EC 2.7.1.90) purified from potato tubers.

Brush border membrane studies

Vesicle preparation. Brush border membrane vesicles (BBMV) were prepared from the proximal and distal small intestine by a CaCl_2 precipitation method as previously described [16]. Purification was assessed through the measurement of sucrase specific activity in the original homogenate and in the final preparation using a coupled glucose 6-phosphate dehydrogenase reaction (Boehringer Mannheim, Dorval, QC, Canada). Sucrase activity was, on average, enriched 14.8 ± 2.6 fold in the BBMV preparations in comparison to whole homogenates, and no difference was seen between groups. The degree of Na,K-ATPase enrichment ranged from 0.6 to 1.4 in all groups, indicating negligible contamination by basolateral membranes.

Transport studies. Glucose uptake studies were carried out at room temperature using the rapid filtration method of Hopfer et al. [17]. Initial uptake rates were measured at an incubation time of 5 s at concentrations of glucose in the incubation medium ranging from 50 $\mu\text{mol/l}$ to 4 mmol/l. To start the reaction, 10 μl of freshly prepared vesicles was added to 50 μl of incubation buffer containing varying concentrations of [^3H]glucose, 100 mmol/l NaSCN, 50 mmol/l mannitol, and 20 mmol/l HEPES; pH 7.5 to start the reaction. The reaction was stopped at 5 s by the addition of 3 ml of icecold stop solution (20 mmol/l HEPES, 100 mmol/l NaCl, and 150 mmol/l mannitol). The resultant mixture was then filtered through a prewetted and chilled 0.45- μm nitrocellulose filter (Millipore) and washed with 9 ml of stop solution. Filters were dissolved in 10 ml scintillation fluid and counted. Non-specific binding of [^3H]glucose to filters was measured by filtration of incubation medium, stop solution, and vesicles without allowing mixing of medium and vesicles, and all results were subsequently corrected. Experiments were performed on at least four different freshly prepared vesicle preparations. Sodium-dependent glucose uptake rates were calculated by subtracting the sodium-independent component measured in the presence of KSCN from the total seen in the presence of NaSCN. Uptake data is reported as nanomoles of glucose per minute per milligram of vesicle protein.

Cycloheximide experiments

To determine if protein synthesis was required for the vanadate-induced effects, rats were injected with a cycloheximide solution (1 g/l in 0.9% NaCl -3 ml/kg) 1 h before vanadate therapy was initiated as described by Cruzoulon and Dandrisse [18]. Control animals received saline alone. Measurements were taken 12 h following administration, and compared with those obtained from diabetic rats and control rats receiving either cycloheximide or vanadate alone.

Statistical analysis

The results were expressed as means \pm SEM. Statistical significance of the data at the 95% confidence level was determined according to ANOVA using the statistical software package SIGMASTAT (Jandel Scientific, San Rafael, Calif., USA). Specific differences were tested using the Student-Newman-

Table 1. Na,K-ATPase activity, kinetic constants for Na-dependent glucose transport in BBMV, and membrane PD in response to vanadate treatment

Group (n)	Na,K-ATPase (nmol · min ⁻¹ · mg protein ⁻¹)	Na-dependent glucose transport (BBMV)		PD (mV)
		J _{max} (nmol · min ⁻¹ · mg protein ⁻¹)	K _m (μmol)	
<i>Jejunum</i>				
Control (6)	134 ± 15	8.7 ± 1.9	667 ± 274	1.6 ± 0.1
Diabetic (6)	220 ± 21 ^a	15.5 ± 2.1 ^a	1181 ± 123	2.2 ± 1 ^a
Group A (4)	205 ± 18 ^a	14.6 ± 4.2 ^a	1023 ± 475	2.1 ± 0.1 ^a
Group B (4)	218 ± 19 ^a	12.8 ± 1.4 ^a	958 ± 190	2.2 ± 0.1 ^a
Group C (4)	155 ± 23 ^b	8.5 ± 1.2 ^b	51 ± 38 ^{a, b}	2.5 ± 0.2 ^a
Group D (4)	194 ± 29 ^a	11.3 ± 1.9	159 ± 41 ^{a, b}	2.5 ± 0.2 ^a
<i>Ileum</i>				
Control (6)	112 ± 15	7.4 ± 2.6	848 ± 157	0.6 ± 0.1
Diabetic (6)	320 ± 16 ^a	14.7 ± 1.4 ^a	819 ± 233	1.2 ± 0.2 ^a
Group A (4)	301 ± 18 ^a	13.8 ± 3.5 ^a	855 ± 248	1.1 ± 0.2 ^a
Group B (4)	313 ± 19 ^a	8.5 ± 1.7 ^b	115 ± 84 ^{a, b}	1.2 ± 0.1 ^a
Group C (4)	109 ± 17 ^b	8.7 ± 1.2 ^b	104 ± 74 ^{a, b}	1.9 ± 0.2 ^a
Group D (4)	157 ± 13 ^a	10.8 ± 1.1	92 ± 57 ^{a, b}	1.4 ± 0.2 ^a

Values are means ± SEM. Treatment groups: Group A, diabetic animals fasted for 12 h; Group B, diabetic animals receiving vanadate for 12 h; Group C, diabetic animals receiving vanadate for 48 h; Group D, diabetic animals treated with vana-

date, 72 h after vanadate has been removed.

^a $p < 0.05$ relative to controls;

^b $p < 0.05$ relative to non-treated diabetics

Keuls analysis as a post hoc test. Curve-fitting and parameter estimation for non-linear relationships were performed with data points weighted inversely to within-sample variances at each concentration [19].

Results

Animal profile. Streptozotocin-treated rats demonstrated elevated blood glucose levels within 24 h of injection (> 18 mmol/l) and remained hyperglycaemic for the duration of the study. Body weight at the time of diabetes induction did not differ between STZ treated (351 ± 12 g) and non-treated (355 ± 14) rats. At 30 days (the time of experimentation) after the induction of diabetes, the average body weight (371 ± 10 g) and blood glucose (> 18 mmol/l) of diabetic rats was significantly different ($p < 0.05$) from those of age-matched control rats (421 ± 14 g; 5.6 ± 0.6 mmol/l).

Rats receiving vanadate in their drinking water showed a rapid decrease in blood glucose levels with-

in 12 h to a slightly hyperglycaemic point where levels stabilized for the duration of vanadate supplementation (Fig. 1A). Removal of vanadate resulted in an increase in blood glucose levels to pre-treatment hyperglycaemic levels within 24 h.

Intact mucosal sheet transport studies. When measured in intact sheets of tissue in Ussing chambers, non-treated diabetic rats demonstrated an increase ($p < 0.02$) in J_{max} for Na-dependent jejunal (61.9 ± 6.1 μAmp/cm²) and ileal (88.6 ± 8.0 μAmp/cm²) glucose transport in comparison with results obtained from age-matched control rats (jejunum: 42.1 ± 5.2; ileum: 20.5 ± 1.5 μAmp/cm²). PD was also increased ($p < 0.05$) in diabetic tissue (jejunum: 2.2 ± 0.2; ileum: 1.2 ± .2 mV) compared with control tissue (jejunum: 1.5 ± 1; ileum: 0.5 ± .1 mV). Upon initiation of vanadate treatment, there was a marked difference in the way the jejunum and ileum responded. As shown in Fig. 1 B, J_{max} for Na-dependent jejunal glucose transport fell gradually and was normalized after 48 h of treatment. In contrast, the ileum re-

Table 2. PFK₁ activity and fructose 2,6-bisphosphate levels in response to vanadate treatment

Group (n)	PFK ₁ activity		Fructose 2,6-bisphosphate (nmol/g dry weight)
	Total activity (nmol · min ⁻¹ · mg protein ⁻¹)	Activity ratio (v0.5/V)	
<i>Jejunum</i>			
Control (6)	83.4 ± 2.1	0.38 ± 0.08	12.7 ± 1.2
Diabetic (6)	40.2 ± 1.2 ^a	0.20 ± 0.06 ^a	6.2 ± 1.5 ^a
Group A (4)	30.2 ± 2.1 ^a	0.16 ± 0.05 ^a	5.4 ± 1.6 ^a
Group B (4)	79.3 ± 1.6 ^b	0.40 ± 0.09 ^b	13.5 ± 1.4 ^b
Group C (4)	71.4 ± 0.7 ^b	0.38 ± 0.08 ^b	13.1 ± 0.9 ^b
Group D (4)	52.9 ± 2.4	0.28 ± 0.09	9.4 ± 1.3
<i>Ileum</i>			
Control (6)	79.5 ± 2.2	0.36 ± 0.07	11.2 ± 1.4
Diabetic (6)	33.0 ± 3.4 ^a	0.24 ± 0.08 ^a	5.2 ± 1.0 ^a
Group A (4)	26.2 ± 3.8 ^a	0.18 ± 0.07 ^a	3.5 ± 1.8 ^a
Group B (4)	60.9 ± 3.5 ^b	0.38 ± 0.06 ^b	12.4 ± 1.3 ^b
Group C (4)	58.4 ± 0.6 ^b	0.38 ± 0.07 ^b	11.8 ± 1.5 ^b
Group D (4)	47.1 ± 3.3	0.30 ± 0.06	9.5 ± 1.2

Values are means ± SEM. Treatment groups: Group A, diabetic animals fasted for 12 h; Group B, diabetic animals receiving vanadate for 12 h; Group C, diabetic animals receiving vanadate for 48 h; Group D, diabetic animals treated with vanadate, 72 h after vanadate has been removed. The total activity of PFK₁ was measured under optimal conditions at pH 8 where the enzyme is not susceptible to allosteric regulation.

The susceptibility of PFK₁ to inhibition by ATP was measured by changes in the activity ratio (v0.5/V) of the activity determined at pH 7 in the presence of 0.5 mmol/l fructose-6-phosphate and 2.5 mmol/l at pH 7.

^a *p* < 0.05 relative to controls; ^b *p* < 0.05 relative to non-treated diabetics

sponded to vanadate supplementation with a rapid reduction in glucose J_{max} within 12 h. Both jejunal and ileal glucose transport was stabilized for the duration of the vanadate treatment (96 h following initiation of treatment). In contrast, PD remained elevated (Table 1) and did not show any decrease in response to vanadate treatment, indicating that inhibition of the sodium electrochemical gradient was probably not responsible for the decrease in sodium-dependent glucose transport.

Removal of vanadate from the drinking water resulted in a rise in blood glucose levels to pre-treatment levels within 24 h (Fig. 1A). In contrast, jejunal glucose transport did not rise to pre-treatment levels until 48 h following removal of vanadate, and ileal glucose transport remained at treatment levels for 72 h following vanadate removal (Fig. 1B). Ileal glucose transport showed a rise to pre-treatment levels 120 h after the removal of vanadate (data not shown).

Na,K-ATPase activity. Vanadate has been shown to inhibit Na,K-ATPase activity in the intestine [20]. Thus, the decrease in intestinal Na-dependent glucose uptake observed in the intact sheets of tissue in the Ussing chambers may have simply resulted from a vanadate-induced inhibition of Na,K-ATPase. To examine this possibility, Na,K-ATPase was measured in tissue homogenant to determine if vanadate treatment was inhibiting enzyme activity. In contrast to the striking changes observed in glucose transport ileal J_{max} after 12 h of vanadate treatment, there was no change seen in Na,K-ATPase enzyme activity for 48 h. While this decrease in Na,K-ATPase activity occurred in parallel with the reduction of glucose transport in the jejunum, it occurred 36 h after ileal glucose transport had normalized. Thus, simple inhibition of Na,K-ATPase activity by vanadate treatment was not likely the cause of the down-regulation of ileal Na-dependent glucose transport.

Following the removal of vanadate supplementation, both jejunal and ileal Na,K-ATPase activity began to increase, approaching pre-treatment levels within 72 h (Table 1).

Brush border membrane vesicle transport studies. Intestinal Na-dependent glucose transport in intestinal mucosal sheets is a function of the activity of glucose carriers on both the brush border membrane SGLT, and the basolateral membrane (GLUT2). We thus measured glucose transport in BBMV, devoid of GLUT2 transport protein, in order to confirm that the vanadate-induced normalization of transport seen in the intact sheets of intestinal tissue was due to a direct effect on SGLT, functional activity. Similar to the results seen in intact mucosal sheets, BBMV isolated from the jejunum and ileum of non-treated diabetic rats demonstrated a significant increase in glucose transport J_{\max} relative to controls (Table 1), while K_m was not different. Table 1 shows the effects of vanadate treatment on BBMV isolated from diabetic rats. Again, as was seen with the intact mucosa sheet studies, jejunal glucose transport J_{\max} in BBMV was normalized 48 h following the beginning of vanadate treatment while ileal J_{\max} was normalized at 12 h. These results indicate that the vanadate-induced transport response occurs at the level of the brush border membrane. In addition, K_m was significantly decreased by vanadate treatment in both jejunum and ileum, indicating a greater affinity of SGLT, for glucose.

Diabetic animals are hyperphagic and vanadate supplementation has previously been shown to reduce food intake [8]. To determine if a reduction in food intake may have contributed to the down-regulation of glucose transport, BBMV were prepared from animals which had been fasted for 12 h. As seen in Table 1, fasting had no effect on intestinal Na-dependent glucose transport. Thus, it is unlikely that a vanadate-induced reduction in food intake was the signal for a down-regulation of glucose transport.

Jejunal and ileal Na-dependent J_{\max} began to increase after 72 h following the removal of vanadate from their drinking water. In contrast, K_m remained significantly decreased.

6-Phosphofructo-1-kinase activity and fructose 2,6-bisphosphate levels. Glucose utilization in the small intestine is reduced in the diabetic state primarily as a result of a decrease in the activity of PFK₁ (fructose-6-phosphate → fructose-1,6-bisphosphate) [4]. PFK₁ itself is activated by micromolar concentrations of fructose 2,6-bisphosphate. In order to determine if oral vanadate treatment also increased the ability of the intestinal enterocyte to utilize glucose, in addition to decreasing the maximal glucose transport rate, we measured PFK₁ activity and fructose

2,6-bisphosphate levels. In accordance with previous studies [4], we found jejunal and ileal enterocyte PFK₁ total activity and activity ratio ($v_{0.05}/V$) to be significantly reduced in diabetic rats in comparison to levels in age-matched control tissue (Table 2). Fructose 2,6-bisphosphate levels were also significantly depressed in diabetic rats (Table 2). Jejunal and ileal PFK₁ total activity and the activity ratio ($v_{0.5}/V$) and fructose 2,6-bisphosphate levels were normalized at 12 h after the initiation of vanadate treatment. To determine if a decrease in food intake was responsible for the effects of vanadate, PFK₁ activity and fructose 2,6-bisphosphate levels were measured in diabetic animals fasted for 12 h. Fasting resulted in a small decrease in both measurements (Table 2). Thus, it is not likely that a reduction in food intake as a consequence of the vanadate treatment was the cause of the increased PFK₁ activity and fructose 2,6-bisphosphate levels.

PFK₁ activity and fructose 2,6-bisphosphate levels began to fall towards pre-treatment levels 72 h following the cessation of vanadate treatment (Table 2).

Cycloheximide studies. To determine if vanadate-induced normalization of intestinal glucose transport and PFK₁ activity was dependent upon protein synthesis, animals were injected with cycloheximide (3 mg/kg) concurrently with vanadate treatment for 12 h. In the presence of cycloheximide, the activation of PFK₁ activity and fructose 2,6-bisphosphate levels by vanadate was blocked in both jejunum and ileum (Table 3). The reduction in ileal glucose transport was also blocked by cycloheximide (Table 3). Diabetic and control animals injected with cycloheximide had no significant changes in either parameter.

Discussion

These results demonstrate that oral vanadate treatment rapidly improves Na-dependent glucose transport in conjunction with an activation of PFK₁ activity in diabetic rat intestine.

It is well documented that vanadate can prevent the changes produced in the small intestine by the induction of experimental diabetes [8] and this effect has generally been considered a secondary consequence of the reduction in hyperglycaemia induced by vanadate [9]. However, the concentration of vanadate used in this study (0.5 mg/ml) stabilized blood glucose levels at a slightly hyperglycaemic level, similar to results obtained by Sekar et al. [21], while PFK₁ activity was normalized along the entire small intestine, and glucose transport was normalized in the jejunum. This would suggest that these changes are not related to blood glucose levels and may reflect a direct cellular effect of vanadate. In addition, both glucose transport and PFK₁ activity remained at

Table 3. Effect of cycloheximide on PFK₁ activity, fructose 2,6-bisphosphate levels, and J_{max} for Na-dependent glucose transport in intact sheets of tissue in Ussing Chambers

Group (n)	Na-dependent glucose transport J _{max} (nmol · min ⁻¹ · mg protein ⁻¹)		PFK ₁ (nmol · min ⁻¹ · mg protein ⁻¹)		Fructose 2,6-bisphosphate (nmol/g dry weight)	
	Jejunum	Ileum	Jejunum	Ileum	Jejunum	Ileum
Control (6)	42.1 ± 5.2	20.5 ± 1.5	83.4 ± 2.1	79.5 ± 2.2	12.7 ± 1.2	11.2 ± 1.4
Control + cycloheximide (4)	54.5 ± 8.9	32.5 ± 5.8	72.8 ± 4.6	61.5 ± 4.2	11.0 ± 1.8	9.8 ± 2.4
Diabetic (6)	61.9 ± 6.1 ^a	88.6 ± 8.0 ^a	40.2 ± 1.3 ^a	33.0 ± 3.4 ^a	6.2 ± 1.5 ^a	5.2 ± 1.0 ^a
Diabetic + cycloheximide (4)	86.1 ± 13.9 ^a	108.3 ± 10.9 ^a	34.5 ± 5.4 ^a	24.5 ± 5.8 ^a	5.1 ± 2.0 ^a	4.6 ± 2.4 ^a
Diabetic + vanadate (4)	56.8 ± 5.8 ^a	39.0 ± 3.5	79.3 ± 2.4	60.9 ± 1.5	13.5 ± 1.4 ^b	12.4 ± 1.3 ^b
Diabetic + vanadate + cycloheximide (4)	62.5 ± 6.1 ^a	85.7 ± 4.8 ^a	38.4 ± 1.5 ^a	31.0 ± 1.0	5.8 ± 2.0 ^a	4.8 ± 1.7 ^a

Values are means ± SEM. Treatments were given for 12 h.

^a *p* < 0.05 relative to controls

^b *p* < 0.05 relative to non-treated diabetics

treatment levels for at least 24 h after the removal of vanadate and subsequent rise in blood glucose levels. Previous studies have shown that vanadate can be stored in bone, kidney, and liver prior to excretion [22]. This cellular retention of vanadate is likely the reason that Na-dependent glucose transport and PFK₁ activity remained at treatment levels following the removal of vanadate treatment, and further supports the hypothesis that vanadate has a direct effect on intestinal enterocytes.

Experimentally produced diabetes is associated with mucosal hypertrophy and increased food intake [23]. The enhanced glucose transport seen in diabetes has been suggested to occur partially as a result of the increased dietary carbohydrate delivery to the intestinal lumen [24]. Although vanadate supplementation suppresses food intake [8], it is unlikely that the results we observed on intestinal Na-dependent glucose transport and PFK₁ activity were due to the anorectic effect of vanadate, as diabetic animals fasted for 12 h did not show an improvement of either parameter.

Conflicting data exists as to the effects of oral vanadate on intestinal glucose transport. We found that the oral dose of vanadate used in this study (0.5 mg/ml) rapidly reduced Na-dependent glucose transport in both jejunum (48 h) and ileum (12 h). This is in conflict with Hajjar et al. [25] who found that oral vanadate (0.3 mg/ml) stimulated D-glucose uptake, although 3-O-methyl glucose uptake was reduced. This may be related to two factors. First, Hajjar et al. [25] used orthovanadate for a period of 30 days, whereas

in this study the effects of vanadium pentoxide over a period of 7 days were examined. These two compounds have different solubilities, toxicity, and speed of elimination [26], which would likely result in different concentrations of intracellular vanadate. Secondly, Hajjar et al. [25] performed the study on normal rats, whereas we examined the effect of oral vanadate on diabetic rats. In our earlier study, we demonstrated that oral vanadate had little effect on normal tissue [8].

Na-dependent glucose transport in the small intestine is dependent upon the potential difference and sodium electrochemical gradient across the brush border membrane. In agreement with previous studies [27], we found an increase in intestinal PD (potential difference) and Na,K-ATPase activity in diabetic animals. Vanadate has been shown to inhibit Na,K-ATPase activity when perfused directly into the intestine at a concentration of 0.01 to 1 mmol/l [20]. Thus, the reduction in Na-dependent glucose transport we observed may have been due to a vanadate-induced dissipation of the sodium electrochemical gradient. However, it is very difficult to determine the intracellular concentrations of vanadate in vivo, as under physiological conditions, internalized VO₃⁻ (a potent Na,K-ATPase inhibitor) is reduced intracellularly to VO²⁺, a species that is very ineffective in inhibiting the enzyme [28]. Previous studies have shown oral vanadate given at a concentration of 0.05 mg/ml to be present in the intestine at a concentration of 49 µg/gm after 6 weeks of treatment [29]. In our study we did not observe a reduction in

Na,K-ATPase enzyme activity until after 48 h of vanadate treatment, which paralleled the changes in jejunal Na-dependent glucose transport, but occurred 36 h after ileal glucose transport had been reduced. This would make it unlikely that the rapid reduction in ileal glucose transport was due to a vanadate-induced inhibition of Na,K-ATPase activity. To confirm this we measured *in vivo* basal intestinal PD, which directly reflects the electrochemical driving (Na,K-ATPase) force for sodium-coupled transport. Basal PD was not affected by vanadate treatment. However, an accurate measurement of not only the total intracellular vanadate concentration, but also its oxidative state would be necessary to determine if orally ingested vanadate is actually inhibiting Na,K-ATPase activity.

Increased glucose transport seen in STZ-induced diabetic rats is accompanied by a premature expression of SGLT, protein and mRNA by enterocytes along the crypt-villus axis in jejunum and ileum [30]. In the jejunum, glucose transporter induction by diet occurs initially in the crypts and progresses up the villus as cells migrate [31]. In our study, the effects of vanadate in the jejunum appear to follow this pattern. Normalization of jejunal Na-dependent glucose transport occurred over a period of 48 h and may have reflected the time it took for cells in the crypt to respond to the signal produced by vanadate supplementation, alter their expression of SGLT, and migrate up the villus. However, the ileal response to vanadate treatment was distinctly different from that of the jejunum. The rapid down-regulation of ileal glucose transport indicates that enterocytes in the ileum have the ability to alter the functional activity of existing glucose transporters. Further work will have to be done to determine if this functional reduction in ileal glucose transport also involves a reduction in SGLT, protein in the brush border membrane.

Vanadate-treated animals demonstrated a large decrease in K_m at both the intact tissue level and at the brush border, indicating an increased affinity of the glucose transporter for glucose in both jejunum and ileum. Glucose transporter affinity may be altered by membrane physical composition [32], and there is evidence that vanadate alters polyunsaturated fatty acid content in membranes, possibly through changes in cholesterol and lipid biosynthesis [33]. Another possibility is that vanadate may alter the expression of the recently described Na-dependent glucose transporter regulatory protein [34]. This regulatory subunit has been proposed to be responsible for mediating nutritional and hormonal signals for the regulation of SGLT, functional activity [34]. The presence of such a regulatory subunit may allow the intestine to rapidly adapt to changing luminal nutrient loads, i.e. increasing the functional expression of a low affinity transporter when large amounts of nutrients are present and switching to a

high affinity transporter when nutrient levels decrease, simply by altering the association between the regulatory subunit and the catalytic SGLT, protein. Oral vanadate reduces food intake [8], thus, a switch to a higher affinity transporter may be a physiological response to decreased luminal nutrients. It is currently unknown, however, if vanadate has any effect on expression of this regulatory subunit.

Intestinal enterocytes have a high glycolytic activity [35]. The reaction catalysed by PFK₁ is the key regulatory step in the control of enterocyte glycolysis and this enzyme itself is activated by micromolar concentrations of fructose 2,6-bisphosphate [36]. Enterocytes from diabetic rats show a decrease in cellular concentrations of fructose 2,6-bisphosphate [37], which is accompanied by a marked reduction in the rate of glycolysis, and a diminished activity of PFK₁ [4]. Our findings are in agreement with these studies. Furthermore, data from this study clearly demonstrate that oral vanadate can rapidly stimulate PFK₁ activity and increase cellular concentrations of fructose 2,6-bisphosphate. The mechanism by which vanadate increases fructose 2,6-bisphosphate concentrations is unknown. The concentration of fructose 2,6-bisphosphate is determined by nutritionally and hormonally induced changes in the activity of the bifunctional enzyme 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase [38]. This enzyme in turn is regulated via phosphorylation/dephosphorylation reactions. It is unlikely that vanadate has a direct action on 6-phosphofructo-2-kinase/fructose 2,6-bisphosphatase, as *in vitro* experiments have shown vanadate inhibits the kinase activity of the purified enzyme without modifying the phosphatase activity [39]. Thus, while there is evidence that vanadate treatment is able to restore cellular fructose 2,6-bisphosphate to normal levels in diabetic rats [40] and to induce fructose 2,6-bisphosphate production in cultured rat hepatocytes [41], the mechanism remains to be elucidated.

The findings in this study are in agreement with those studies showing that vanadate can stimulate overall intestinal glucose metabolism [25, 42]. In fact, it has been suggested previously that there is a relationship between glucose metabolism and glucose transport in the intestine [43]. Data from this study showing changes in PFK₁ activity and fructose 2,6-bisphosphate levels to precede and parallel glucose transport changes also suggests a possible relationship.

In conclusion, oral vanadate treatment rapidly reduces glucose transport and restores 6-phosphofructo-1-kinase activity and fructose 2,6-bisphosphate levels to normal values in the intestine of diabetic rats. This would have the overall effect of increasing glucose utilization within the enterocyte and thus decreasing overall glucose absorption. In addition, both transport and PFK₁ activity remain at treat-

ment levels following the removal of vanadate. Thus, oral vanadate treatment can have prolonged beneficial effects on intestinal function and may prove useful as oral adjunctive diabetic therapy.

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