Vanadate Action on Renal Phosphate Transport (43799)

AMIT N. SANGHVI, DAWN K. STEENBERGEN, SCOTT A. SEIFERT, STEPHEN A. KEMPSON,[1] AND DANIEL E. PEAVY

Department of Physiology and Biophysics, Indiana University School of Medicine, Indianapolis, Indiana 46202–5120

Abstract. **Insulin stimulates reabsorption of phosphate (Pi) in the renal proximal tubule. Previous studies have shown that vanadate can mimic the action of insulin on various tissues. In the present study, we tested the action of vanadate on renal Pi transport both in control rats and in rats made diabetic by injection of streptozotocin. Vanadate was administered orally for 4 days by inclusion in drinking water (0.7 mg/ml). By the 4th day, vanadate treatment of control rats did not change acid-base status, plasma glucose or the filtered load of Pi, but the urinary excretion of Pi was reduced to 2.5 ± 0.9 compared with 17.6 ± 3.5 μmol/mg creatinine ($P < 0.02$) in untreated control rats. However, Na^+/Pi cotransport by isolated brush border membrane vesicles was not different between the two groups. Findings in parathyroidectomized rats were similar. By the 4th day of vanadate treatment of diabetic rats, there was reversal of polyuria, polydipsia and hyperglycemia with no change in acid-base status. The filtered load of Pi was decreased by vanadate, and urinary Pi excretion also tended to decrease but not significantly. The values for Pi excretion were 21.4 ± 7.6 in vanadate treated diabetics and 36.1 ± 4.5 μmol/mg creatinine in untreated diabetics. In contrast to vanadate, daily injections of insulin did not change the filtered load of Pi but reduced urinary Pi excretion in diabetic rats to 15.6 ± 2.2 μmol/mg creatinine ($P < 0.02$). These findings suggest that vanadate stimulated tubular Pi reabsorption in control rats but not in diabetic rats. Vanadate treatment of diabetic rats may tend to decrease tubular Pi reabsorption in contrast to the action of insulin.**

[P.S.E.B.M. 1994, Vol 207]

Vanadium occurs in a variety of plants and animals, many of which are ingested by humans. It is an essential nutrient in the rat (1). In solution, it forms the vanadate anion which is absorbed from intestine and eliminated principally by the kidneys (2, 3). Most mammalian tissues, including liver and bone, contain traces of vanadium but the highest concentration is in the renal cortex (1, 2). Thus the kidneys may be a major site of vanadate action. The renal effects of vanadate include a mixture of hemodynamic and tubular actions which appear to be species-specific. Cardiovascular and vasoconstrictor effects predominate in cats and dogs, whereas a marked diuresis and natriuresis occur in acute studies in rats (2). Neither acute (4) nor chronic (5, 6) treatment of rats with vanadate produced significant changes in glomerular filtration rate. Data from acute studies suggest that the diuresis in the rat is due primarily to inhibition of Na^+/K^+-ATPase pump activity in most nephron segments (1). A full understanding of the renal effects of vanadate has not been reached because multiple mechanisms may be involved.

In recent years, there has been considerable interest in the insulinomimetic effects of vanadate (7, 8). This stems from the fact that when vanadate is given to diabetic animals, it restores blood glucose values to normal within a few days (7, 9–12) and the euglycemic state can be maintained for as long as 9–12 weeks (10, 13), even after vanadate withdrawal (14). Several diabetes-induced physiological and metabolic changes were shown to be reversed by vanadate administration, including glucosuria (10), impaired cardiac per-

[1] To whom requests for reprints should be addressed at Medical Sciences 374, 635 Barnhill Drive, Indianapolis, IN 46202–5120.

Received December 7, 1993. [P.S.E.B.M. 1994, Vol 207]
Accepted May 23, 1994.

0037-9727/94/2071-0110$10.50/0

formance (15), impaired glucose disposal (9–11, 14), hepatic glucose production and hepatic PEPCK activity (11). *In vitro,* vanadate (usually as sodium orthovanadate) has been shown to produce several insulin-like actions in a variety of target tissues, including adipocytes (16–20), hepatocytes (21–24), and skeletal muscle (25).

Results from a number of studies have suggested that insulin may have a physiologic role in the acute renal handling of inorganic phosphate (Pi). Insulin decreases renal Pi excretion in fasted rats (26), and reverses or prevents the phosphaturic effect of parathyroid hormone (27, 28). The hormone also appears to play a role in the acute renal adaptation to dietary Pi restriction (29, 30). *In vitro,* insulin stimulates Pi uptake by cultured opossum kidney cells (31) and stimulates Pi uptake by proximal tubular brush border membrane vesicles (32). In the present study, we determined if vanadate stimulated renal Pi transport in normal and diabetic rats.

Materials and Methods

Animals. Male rats of the Sprague-Dawley strain initially weighing 125–149 g were obtained from Harlan Industries (Indianapolis, IN). Surgically parathyroidectomized (PTX) rats of the same strain and weight range were purchased from Charles River (Wilmington, MA), as in previous studies (33). Animals were maintained on a 12-hr light:dark cycle, and were given free access to food and fluids at all times, except for the 12-hr period prior to induction of diabetes, when food was withdrawn. Upon arrival, animals were placed in standard wire-bottom cages and were provided a pellet rodent food (Rodent Laboratory Chow #5001; Ralston Purina, St. Louis, MO) for 2–3 days to adapt to the new environment. At the start of an experiment (Day 0), animals were housed individually in metabolic cages and a powdered form of the same diet was provided for the duration of the experiment. Successful PTX was indicated by a lower urinary Pi excretion compared to intact controls on the 5th day after surgery. Pi excretion was 2 ± 1 in PTX rats compared with 41 ± 4 (mean ± SE, $n = 4$) μmol/24 hr/100 g in intact rats ($P < 0.005$).

Induction of Diabetes. Diabetes was induced in some of the rats on Day 4 of the experiment, following an overnight fast. Animals were lightly anesthetized with ether and injected via the saphenous vein with 60 mg/kg body wt of freshly prepared streptozotocin (Sigma) which was dissolved in ice-cold 144 m*M* NaCl, 5 m*M* trisodium citrate buffer, pH 4.5. Control animals received vehicle only. Induction of diabetes was initially confirmed by urinary glucose measurements using Keto-Diastix (Ames).

Vanadate and Insulin Treatment. On Day 7 of the experiment, control and diabetic rats were divided into the following groups: (i) control, no treatment, (ii) control, vanadate treated; (iii) diabetic, no treatment; (iv) diabetic, vanadate treated; and (v) diabetic, insulin treated. All five groups were processed in parallel. Rats were treated with vanadate by providing them with drinking water containing sodium orthovanadate (Na_3VO_4, Fisher Scientific) at 0.7 mg/ml and 85 m*M* NaCl. Inclusion of NaCl was reported to reduce possible toxic effects of vanadate (6, 15). All other animals in the experiment were given drinking water containing 85 m*M* NaCl only. Vanadate solutions were prepared fresh every 2 days. Insulin treatment consisted of a daily subcutaneous injection of 1.6 U/100 g body wt of a depot form of insulin (Protamine, Zinc, and Iletin I, Eli Lilly & Co., Indianapolis, IN). These injections occurred between 8:00 and 10:00 AM.

Sample Collection. Body weight and food and water intake were measured each day and urine was collected every 24 hr. On the morning of Day 11, animals were anesthetized with ether, and a blood sample was drawn from the descending aorta using a heparinized syringe. The syringe was sealed and blood gases were determined immediately. In some groups, the kidneys were excised and brush border membrane vesicles were prepared from the renal cortex using magnesium precipitation, as described previously in detail (30, 33).

Transport Studies. The identity and purity of the isolated membrane vesicles were determined routinely by measuring the activity of alkaline phosphatase, a brush border membrane enzyme. The purity was not changed by vanadate treatment. In untreated control rats, for example, alkaline phosphatase activity was enriched 9-fold in the brush border membrane fraction compared to the starting homogenate. In vanadate treated controls, the enrichment was 11-fold.

Isolated brush border membrane vesicles, suspended in 300 m*M* mannitol, 5 m*M* Tris (adjusted to pH 7.4 with Hepes), were used immediately for measurement of Na^+-dependent transport of Pi and proline by the rapid filtration procedure described elsewhere in detail (30, 33). Briefly, the membrane vesicles (0.1 mg protein/tube) were added to uptake medium containing (final concentrations) 100 m*M* NaCl, 100 m*M* mannitol, 5 m*M* Tris-Hepes (pH 7.4), and either 0.1 m*M* $K_2H^{32}PO_4$ or 0.05 m*M* L-[^{3}H]proline. The uptake at 20°C was terminated by addition of ice-cold stopping solution followed by filtration through Millipore filters (0.65 μm). Na^+-independent uptake was determined by replacing NaCl in the uptake medium with KCl. Na^+-independent uptake at 10 sec accounted for less than 5% of the total solute uptake measured in the presence of Na^+. All uptakes were corrected for nonspecific binding of radioisotopes to filters and membranes (30, 33).

Other Assays. Blood gases and pH were ana-

lyzed with an IL Micro 13–03 pH/blood gas analyzer (Instrumentation Laboratory, Lexington, KY). The blood remaining was centrifuged and the plasma was removed and stored in aliquots at −20°C. The colorimetric methods described previously (30, 33) were used to determine Pi, creatinine, and glucose content in plasma and urine samples, and protein content in isolated membrane vesicles. Vanadate at concentrations up to 10 m*M* did not interfere with these colorimetric methods.

Differences between treated and nontreated groups were analyzed with the Student's *t* test for group comparisons. Values of $P > 0.05$ were considered not significant.

Results

Vanadate treatment of control rats for 4 days produced no change in acid-base status compared with untreated controls (Table I). Intake of food and water was decreased significantly, but not dramatically, by vanadate, but body weight and blood glucose were not changed (Table I), as reported previously (15). It is important to note that a fasting response, which changes renal Pi transport, is not induced unless rats consume less than 3 g food each day (33). Fasting also produces a rapid decrease in body weight which is readily detected. Urine volume was decreased by vanadate and plasma Pi also was decreased. Vanadate tended to increase GFR but the change was not statistically significant, in agreement with previous reports (5, 6). The filtered load of Pi was similar in the two groups but urinary Pi excretion (expressed relative to creatinine excretion) was decreased significantly by vanadate on Day 11 (Table I). Studies on the time course showed that the change in Pi excretion developed slowly and was not apparent until Day 10 (Fig. 1), the 3rd day of vanadate treatment. The findings at Day 11 suggest indirectly that an increase in tubular reabsorption of Pi occurred in response to oral vanadate.

Table I. Control Rats: Acid-Base Status, Food/Water Intake and Renal Function at End of Study (Day 11)[a]

	Untreated	+ Vanadate
Acid-Base status		
Blood pH	7.37 ± 0.03	7.35 ± 0.03
P_{CO2} (mm Hg)	42.6 ± 4.4	35.5 ± 4.5
HCO_3 (mEq/l)	24.0 ± 1.0	19.5 ± 3.5
Food/Water intake		
Water (ml/24 hr)	41 ± 3	27 ± 2[b]
Food (g/24 hr)	21 ± 1	17 ± 1[b]
Body wt (g)	219 ± 6	206 ± 6
Blood glucose (mg/dl)	148 ± 4	156 ± 7
Renal function		
Urine volume (ml/24 hr)	21 ± 3	11 ± 1[b]
Plasma Pi (m*M*)	1.93 ± 0.14	1.30 ± 0.10[b]
GFR (ml/24 hr/100 g)	604 ± 150	1088 ± 215
Filtered load Pi (mmol/24 hr/100 g)	1.19 ± 0.32	1.37 ± 0.24
Urine Pi (μmol/mg Cr)	17.6 ± 3.5	2.5 ± 0.9[b]

[a] Treatment of normal rats with vanadate was carried out as described in Materials and Methods. Data are mean ± SE from 8 rats in each group. Cr, creatinine.
[b] Significantly different ($P < 0.02$) compared with untreated group.

Although the filtered load of Pi was not changed significantly by vanadate, the variability in plasma Pi and GFR (Table I) raises the question that a fall in plasma Pi might be the primary cause of the decrease in urinary Pi excretion in vanadate-treated rats. This was addressed by repeating the study in an additional group of rats which had undergone PTX to eliminate endogenous parathyroid hormone, an important inhibitor of renal Pi transport (27, 28). In brief, the findings were similar to those shown in Table I for intact rats. Although vanadate-treated PTX rats consistently drank less of the vanadate solution (10 ± 1 ml on Day 11) compared with the vanadate-treated intact rats (Table I), the decrease in urinary Pi excretion was reproduced. The values for Pi excretion on Day 11 were 1.7 ± 0.2 in untreated rats compared with 0.4 ± 0.1 (mean ± SE, 4 rats/group) μmol/mg creatinine ($P < 0.02$) in the vanadate-treated group. The PTX rats, unlike the intact rats showed no significant change in plasma Pi after vanadate treatment (untreated, 1.87 ± 0.14 m*M*; vanadate, 2.22 ± 0.51 m*M*). Variability in the GFR was less marked (untreated, 905 ± 97; vanadate 867 ± 122 ml/24 hr/100 g) and was not different between the two groups. Thus, as in intact rats (Table I), vanadate produced no significant change in the filtered load of Pi (untreated, 1.7 ± 0.3; vanadate, 1.9 ± 0.2 mmol/24 hr/100 g). This supports the previous conclusion that oral vanadate may increase tubular reabsorption of Pi.

Treatment of diabetic rats with vanadate or insulin

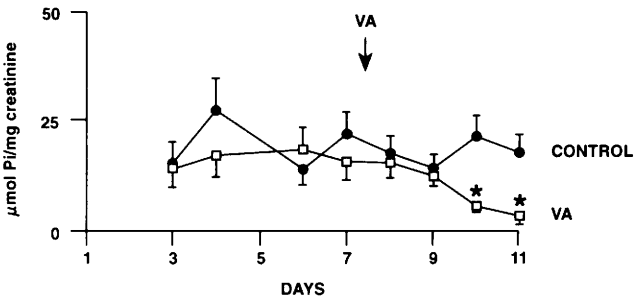

Figure 1. Urinary excretion of phosphate in nondiabetic rats given oral vanadate (VA) or vehicle (control). Vanadate treatment was begun after Day 7. Each point represents the mean ± SE from 8 rats. *Significantly different ($P < 0.005$, group *t* test) compared with untreated controls.

Table II. Diabetic Rats: Acid-Base Status, Food/Water Intake and Renal Function at End of Study (Day 11)[a]

	Untreated	Vanadate	Insulin
Acid-Base status			
Blood pH	7.48 ± 0.03	7.42 ± 0.06	7.42 ± 0.01
P_{CO2} (mm Hg)	30.0 ± 3.3	36.8 ± 4.3	37.8 ± 1.6
HCO_3 (mEq/l)	22.4 ± 1.0	23.9 ± 0.6	25.0 ± 1.6
Food-Water intake			
Water (ml/24 hr)	216 ± 14	33 ± 4[b]	64 ± 10[b]
Food (g/24 hr)	29 ± 1	15 ± 1[b]	23 ± 3
Body wt (g)	179 ± 6	159 ± 7[b]	211 ± 7[b]
Blood glucose (mg/dl)	491 ± 32	167 ± 43[b]	144 ± 29[b]
Renal function			
Urine volume (ml/24 hr)	182 ± 11	14 ± 3[b]	34 ± 4[b]
Plasma Pi (m*M*)	1.58 ± 0.13	1.02 ± 0.17[b]	1.86 ± 0.14
GFR (ml/24 hr/100 g)	1994 ± 367	631 ± 153[b]	1007 ± 190[b]
Filtered load Pi (mmol/24 hr/100 g)	3.26 ± 0.71	0.74 ± 0.21[b]	2.02 ± 0.44
Urine Pi (μmol/mg Cr)	36.1 ± 4.5	21.4 ± 7.6	15.6 ± 2.2[b]

[a] Treatment of diabetic rats with vanadate or insulin was carried out as described in Materials and Methods. Cr, creatinine. Data are mean ± SE from 9–11 rats in each group.
[b] Significantly different ($P < 0.03$) compared with untreated group.

produced no change in acid-base status compared with untreated diabetics (Table II). Both vanadate and insulin reduced water intake of diabetic rats to the level seen in the controls (Table I). Vanadate also produced a significant fall in food consumption which may contribute to the decreased body weight in this group compared with untreated diabetics. However, all vanadate treated rats consumed far more than 3 g food/day (Table II), so a fasting response can be excluded, as with control rats. Insulin treatment, in contrast, did not change food intake, and the body weight of this group was increased significantly compared with the untreated diabetics (Table II). Both vanadate and insulin decreased the blood glucose level and urine volume of diabetic rats to the range observed in the controls (Table I). Normalization of blood glucose in diabetic animals by vanadate has been reported previously (7, 9–12). Based on these physiological changes, the oral dose of vanadate received by the diabetic rats was as potent as injections of insulin (Table II). Plasma Pi of diabetic rats was decreased by vanadate, as in controls, but not by insulin (Table II). Vanadate, but not insulin, decreased the filtered load of Pi compared with the untreated diabetics (Table II). Urinary Pi excretion was decreased by insulin, compared with the untreated group. Vanadate produced a similar trend by Day 11, but the difference was not statistically significant (Table II).

As expected, urinary Pi excretion tended to increase in all groups following injection of streptozotocin and remained elevated about 2-fold in untreated diabetics throughout the rest of the time course (Fig. 2). Treatment with exogenous insulin reversed this change and returned urinary Pi excretion to the level prior to injection of streptozotocin. Urinary Pi excretion in insulin treated diabetics was consistently lower compared with untreated diabetics during the period from Day 8 to Day 11 and the difference reached statistical significance on Day 8 and 11 (Fig. 2). In marked contrast to its action in control rats (Table I), vanadate treatment of diabetic rats produced a transient increase in urinary Pi excretion on Day 8–10, but the changes were not statistically significant compared with the untreated diabetics throughout the time course of this study (Fig. 2).

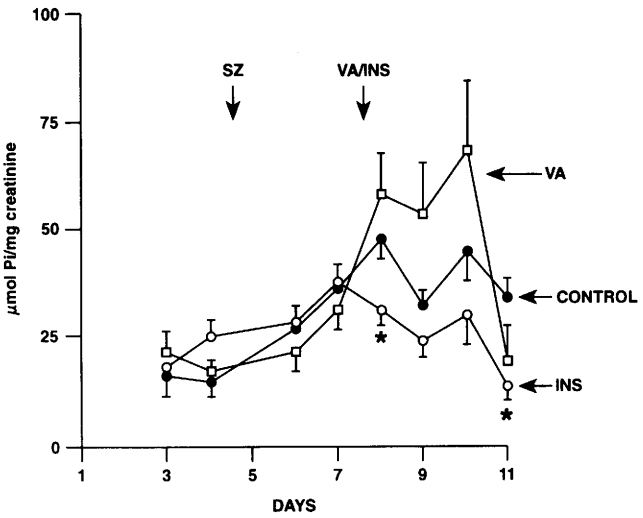

Figure 2. Urinary excretion of phosphate in rats made diabetic by streptozotocin (SZ) injection on Day 4. After Day 7 the rats were given either oral vanadate (VA), daily insulin injections (INS), or left untreated (control). Each point is the mean ± SE from 9–11 rats. *Significantly different ($P < 0.001$, group t test) compared with untreated controls.

In an attempt to identify the mechanism and site of action of vanadate on renal tubular transport of Pi in

control rats (non-PTX), Na^+/Pi cotransport was measured in isolated brush border membrane vesicles derived from the renal proximal tubule. This nephron segment is the site where most of the filtered phosphate is reabsorbed. The integrity of the vesicle preparations was confirmed by the characteristic overshoot in Pi uptake at 10 sec compared with 120 min, the equilibrium point (Fig. 3). Furthermore, the Pi uptake at 10 sec was markedly Na^+-dependent. In the absence of Na^+, the values for Pi uptake were only 30 ± 2 and 28 ± 7 pmol/mg/10 sec in untreated and vanadate treated groups, respectively. Na^+/Pi cotransport at 10 sec, the initial phase of uptake, was not different between untreated and vanadate treated groups (Fig. 3). Na^+/proline cotransport also was not changed significantly by vanadate. These findings indicate that the stimulatory effect of vanadate on Pi reabsorption in control rats cannot be detected *in vitro* using isolated membrane vesicles.

Discussion

The present study involved chronic treatment of rats with vanadate and focused specifically on whether vanadate could stimulate renal tubular reabsorption of filtered Pi. In the control (nondiabetic) rats, both intact (Fig. 1) and PTX, oral vanadate decreased the urinary excretion of Pi, a finding which contrasts with acute studies in which intravenous infusion of vanadate into rats caused an increase in Pi excretion (4, 34). Thus, with regard to renal Pi transport in control rats, the results of vanadate treatment appear to depend on time of treatment and, possibly, route of administration. The decrease in urinary Pi excretion observed in the present chronic study is a change which parallels the known antiphosphaturic action of insulin (26). Insulin stimulates the Na^+/Pi cotransport system in the brush border membrane of the proximal tubule (32), but, in the present study, vanadate treatment produced no detectable change in this Pi transport system (Fig. 3). It is possible that vanadate action on the proximal tubule *in vivo* is not detectable in isolated brush border vesicles. For example, *in vivo* filtered vanadate may compete with Pi for the Na^+/Pi cotransporter in the brush border membrane or vanadate may inhibit Na^+/K^+-ATPase in the basolateral membrane and collapse the Na^+ gradient. Neither of these effects would be detected in isolated brush border membrane vesicles. However, both these actions would produce an increase in urinary Pi excretion, and this did not occur in the present study. Vanadate may stimulate Na^+/Pi cotransport indirectly by changing cellular metabolism, but, again, this effect would not be detected in isolated membrane vesicles. Another indirect effect of vanadate *in vivo* may be to interfere with the renal action of parathyroid hormone so that the normal inhibitory action of this hormone on tubular Pi reabsorption is blocked. This is unlikely, however, because the effect of vanadate was reproduced in PTX rats. Furthermore, if this was the major mechanism of vanadate action, then similar findings would be expected in both control and diabetic groups. This did not occur. In summary, based on the data presented here, it is not possible to determine the site and the cellular mechanism of vanadate action on Pi transport in the nephron.

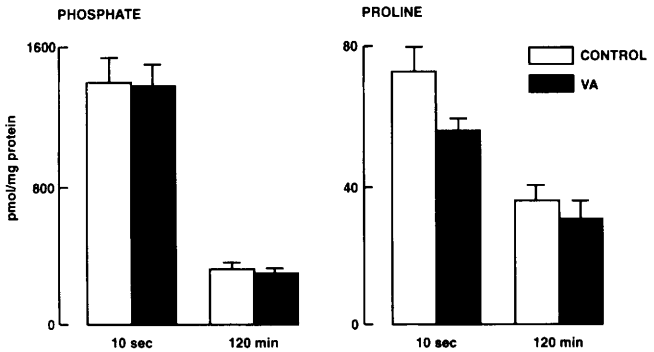

Figure 3. Sodium-dependent solute transport in renal brush border membrane vesicles isolated from nondiabetic rats on Day 11. The rats were either untreated (control) or given oral vanadate (VA). Data are mean ± SE from three different membrane preparations.

The findings on vanadate action *in vivo* in control rats complement studies on vanadate action in an *in vitro* system using cultured renal epithelial OK cells (35). Extracellular vanadate (0.05–1.00 m*M*) produced dose-dependent stimulation of Na^+/Pi cotransport by these cells. The stimulation of Pi uptake was correlated with stimulation of protein phosphorylation either by vanadate stimulation of tyrosine kinase or by vanadate inhibition of phosphotyrosyl-protein phosphatase.

Unlike its action in the control group of rats, vanadate treatment of diabetic rats for the same period of time produced variable, but not significant, changes in urinary Pi excretion (Fig. 2). Plasma glucose in vanadate-treated diabetic rats was decreased significantly and was in the range of values found in the nondiabetic controls. In contrast, treatment of diabetic rats with insulin normalized both plasma glucose and urinary Pi excretion (Tables I and II). Restoration of normoglycemia with no significant change in urinary Pi excretion in vanadate treated diabetics suggests, indirectly, that the phosphaturia in untreated diabetic rats (Fig. 2) is not due solely to the osmotic effect of a high glucose concentration in the glomerular filtrate. It has been reported that normalization of plasma glucose by vanadate does not involve an increase in plasma insulin, suggesting that insulin target tissues are the site of vanadate action (36). The fall in plasma glucose in vanadate treated diabetic rats is due in part to increased

glucose uptake in liver and muscle (36). The kidney is also a target for insulin, and long-term oral vanadate treatment has been reported to prevent the increase in urinary excretion of albumin in diabetic rats, suggesting an improvement in diabetic kidney function (6). The present study, however, shows clearly that vanadate did not reproduce the action of insulin on renal Pi transport in diabetic rats.

The reason for the different responses of control and diabetic rats to vanadate is not understood at the present time. It should be noted, however, that vanadate does not produce a full range of insulin-like effects in every system that has been examined (22, 25, 37), suggesting a divergence between the two agents at some point in the signal transduction pathway. The dissociation of vanadate action from insulin action in diabetic rats may make vanadate a useful tool to help understand the cellular mechanism and the role of insulin regulation of renal Pi transport.

We thank Dr. George Tanner for helpful comments on the manuscript. This work was published in part in abstract form in FASEB J **7**:A12, 1993. These studies were supported by National Institutes of Health Grant DK32148 and General Medical Research Funds from the Department of Veterans Affairs.

1. Nechay BR. Mechanism of action of vanadium. Ann Rev Pharmacol Toxicol **24**:501–524, 1984.
2. Grantham JJ. The renal sodium pump and vanadate. Am J Physiol **239**:F97–F106, 1980.
3. Phillips TD, Nechay BR, Heidelbaugh ND. Vanadium: Chemistry and the kidney. Fed Proc **42**:2969–2973, 1983.
4. Westenfelder C, Hamburger RK, Garcia ME. Effect of vanadate on renal tubular function in rats. Am J Physiol **240**: F522–F529, 1981.
5. Higashino H, Bogden JD, Lavenhar MA, Bauman JW, Hirotsu T, Aviv A. Vanadium, Na-K-ATPase, and potassium adaptation in the rat. Am J Physiol **244**:F105–F111, 1983.
6. Nakazawa A, Igarishi K, Tani N, Momotsu T, Ito S, Shibata A. Effect of vanadate on renal function in rats with streptozotocin-induced diabetes. J Diabetic Comp **5**:179–180, 1991.
7. Shechter Y. Insulin-mimetic effects of vanadate. Possible implications for future treatment of diabetes. Diabetes **39**:1–5, 1990.
8. Cros G, Mongold J-J, Serrano J-J, Ramanadham S, McNeill JH. Effects of vanadyl derivatives on animal models of diabetes. Mol Cell Biochem **109**:163–166, 1992.
9. Meyerovitch J, Farfel Z, Sack J, Shechter Y. Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats. Characterization and mode of action. J Biol Chem **262**:6658–6662, 1987.
10. Brichard SM, Okitolonda W, Henquin JC. Long-term improvement of glucose homeostasis by vanadate treatment in diabetic rats. Endocrinology **123**:2048–2053, 1988.
11. Blondel O, Simon J, Chevalier B, Portha B. Impaired insulin action but normal insulin receptor activity in diabetic rat liver: Effect of vanadate. Am J Physiol **258**:E459–E467, 1990.
12. Rossetti L, Giaccari A, Klein-Robbenhaar E, Vogel LR. Insulinomimetic properties of trace elements and characterization of their *in vivo* mode of action. Diabetes **39**:1243–1250, 1990.
13. Sakurai H, Tsuchiya K, Nukatsuka M, Sofue M, Kawada J. Insulin-like effect of vanadyl ion on streptozotocin-induced diabetic rats. J Endocrinol **126**:451–459, 1990.
14. Pederson RA, Ramanadham S, Buchan AMJ, McNeil JH. Long-term effects of vanadyl treatment on streptozotocin-induced diabetes in rats. Diabetes **38**:1390–1395, 1989.
15. Heyliger CE, Tahiliani AG, McNeill JH. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science **227**:1474–1477, 1985.
16. Dubyak GR, Kleinzeller A. The insulin-mimetic effects of vanadate in isolated rat adipocytes. Dissociation from effects of vanadate as a (Na^+-K^+)ATPase inhibitor. J Biol Chem **255**: 5306–5312, 1980.
17. Shechter Y, Karlish JD. Insulin-like stimulation of glucose oxidation in rat adipocytes by vanadyl (iv) ions. Nature **284**:556–558, 1980.
18. Duckworth WC, Solomon SS, Liepnieks J, Hamel FG, Hand S, Peavy DE. Insulin-like effects of vanadate in isolated rat adipocytes. Endocrinology **122**:2285–2289, 1988.
19. Mooney RA, Bordwell KL, Luhowskyj S, Casnellie JE. The insulin-like effect of vanadate on lipolysis in rat adipocytes is not accompanied by an insulin-like effect on tyrosine phosphorylation. Endocrinology **124**:422–429, 1989.
20. Fantus IG, Ahmad F, Deragon G. Vanadate augments insulin binding and prolongs insulin action in rat adipocytes. Endocrinology **127**:2716–2725, 1990.
21. Gomez-Foix AM, Rodriguez-Gil JE, Fillat C, Guinovart JJ, Bosch F. Vanadate raises fructose 2,6-biphosphate concentrations and activates glycolysis in rat hepatocytes. Biochem J **255**:507–512, 1988.
22. Jackson TK, Salhanick AI, Sparks JD, Sparks CE, Bolognino M, Amatruda JM. Insulin-mimetic effects of vanadate in primary cultures of rat hepatocytes. Diabetes **37**:1234–1240, 1988.
23. Miralpeix M, Decaux J-F, Kahn A, and Bartrons R. Vanadate induction of L-type pyruvate kinase mRNA in adult rat hepatocytes in primary culture. Diabetes **40**:462–464, 1991.
24. Rodriguez-Gil JE, Gomez-Foix AM, Fillat C, Bosch F, Guinovart JJ. Activation by vanadate of glycolysis in hepatocytes from diabetic rats. Diabetes **40**:1355–1359, 1991.
25. Clark AS, Fagan JM, Mitch WE. Selectivity of the insulin-like action of vanadate on glucose and protein metabolism in skeletal muscle. Biochem J **232**:273–276, 1985.
26. Lau K, Guntupalli J, Eby B. Effects of somatostatin on phosphate transport: Evidence for the basal role of insulin. Kidney Int **24**:10–15, 1983.
27. Guntupalli J, Rogers A, Bourke E. Effect of insulin on renal phosphorus handling in the rat: Interaction with PTH and nicotinamide. Am J Physiol **249**:F610–F618, 1985.
28. Allon M, Dass P, Bourke E, Guntupalli J. Prevention of the phosphaturic action of PTH by insulin. (abstract) Kidney Int **31**:341, 1987.
29. Allon M, Rodriguez M, Llach F. Insulin in the acute renal adaptation to dietary phosphate restriction in the rat. Kidney Int **37**:14–20, 1990.
30. Abraham MI, Woods RE, Breedlove DK, Kempson SA. Renal adaptation to low phosphate diet in diabetic rats. Am J Physiol **262**:F731–F736, 1992.
31. Abraham MI, McAteer JA, Kempson SA. Insulin stimulates phosphate transport in opossum kidney epithelial cells. Am J Physiol **258**:F1592–F1598, 1990.
32. Hammerman MR, Rogers S, Hansen VA, Gavin JR. Insulin

stimulates Pi transport in brush border vesicles from proximal tubular segments. Am J Physiol **247**:E616–E624, 1984.
33. Kempson SA. Effect of metabolic acidosis on renal brush border membrane adaptation to low phosphorus diet. Kidney Int **22**:225–233, 1982.
34. Day H, Middendorf D, Lukert B, Heinz A, Grantham J. The renal response to intravenous vanadate in rats. J Lab Clin Med **96**:382–395, 1980.
35. Caverzasio J, Bonjour J-P. Tyrosine phosphorylation selectively regulates renal cellular phosphate transport. Evidence that it mediates the stimulatory effect of insulin-like growth factor-I. Endocrinology **130**:373–380, 1992.
36. Miralpeix M, Carbello E, Bartrons R, Crepin K, Hue L, Rousseau GG. Oral administration of vanadate to diabetic rats restores liver 6-phosphofructo-2-kinase content and mRNA. Diabetologia **35**:243–248, 1992.
37. Bosch F, Arino J, Gomex-Foix AM, Guinovart JJ. Glycogenolytic, noninsulin-like effects of vanadate on rat hepatocyte glycogen synthase and phosphorylase. J Biol Chem **262**:218–222, 1987.