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Effect of Vanadate on Amino Acid Transport in Rat Jejunum (42493)

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Abstract. Vanadate has been reported to inhibit ($Na^+ + K^+$)-ATPase of many cells and in some systems to stimulate adenylate cyclase. Since intestinal transport is influenced by these enzymes, we studied the effects of varying concentrations of orthovanadate (VO_4^-) on alanine transport in the *in vitro* rat jejunum. At the higher concentrations tested (10^{-3} and $10^{-2}M$) vanadate had a ouabainlike action on alanine transport. It decreased the mucosal-to-serosal flux and the influx of alanine into the intestinal epithelium and it caused a reduction of ($Na^+ + K^+$)-ATPase activity of basolateral membranes. The relatively lower vanadate concentration of $10^{-4}M$ increased the influx and the efflux of alanine across the mucosal border of the jejunum. The increase was associated with elevation of cyclic AMP in the intestinal mucosa. The studies suggest the presence of a dual action of vanadate on amino acid transport, a stimulatory effect at low concentration, due to increased adenylate cyclase activity, and an inhibitory effect at higher concentrations, due to a decreased activity of ($Na^+ + K^+$)-ATPase. © 1987 Society for Experimental Biology and Medicine.

Pentavalent vanadium (vanadate) is recognized as a potent inhibitor of Na-K-ATPase. Inhibition of this enzyme has been demonstrated in purified enzyme preparations (1–3), isolated cells (4–6) and epithelia (7–10). Vanadate also inhibits many other enzymes, including Ca-ATPase (11, 12), H-ATPase (13), acid phosphatase (14), alkaline phosphatase (15), and dynein Mg-ATPase (16). Surprisingly, however, and only in cardiac, fat, and liver cells, vanadate stimulates adenylate cyclase (17–21) and causes elevations in cAMP levels. In a recent publication (22), we found that vanadate, depending on the concentration, can stimulate secretion and inhibit absorption of water and ions in the rat jejunum. These effects were thought to be due respectively to stimulation of adenylate cyclase and inhibition of ($Na^+ + K^+$)-ATPase.

Since amino acid transport across the intestinal epithelium is influenced by both ($Na^+ + K^+$)-ATPase (23) and adenylate cyclase (24, 25), we studied the effect of vanadate on transport of alanine across the rat jejunum.

Materials and Methods. *Animals.* Male Sprague–Dawley rats weighing 250–300 g were used. They were kept under controlled conditions (12:12 light:dark room, 25°C) and fed Purina Lab chow and water *ad libitum.* The effect of vanadate was studied in four series of experiments as described below. The rats were killed by decapitation and segments of jejunum were resected and cut longitudinally along their mesenteric lines into flat sheets that were incubated in Krebs–phosphate buffer (26) and oxygenated with bubbling humidified O^2.

Unidirectional transmural fluxes of alanine. In the first series of experiments, the jejunal segments were mounted in Lucite chambers. The chambers and the method of flux measurement were similar to those described by Munck (27) using a method similar to the Ussing–Zerahn technique. Mucosal-to-serosal (Jms) and serosal-to-mucosal (Jsm) fluxes were determined simultaneously in four segments of intestine. Two tissues were bathed by Krebs–phosphate buffer (controls) and the other two were exposed to vanadate added to the buffer bathing their serosal side. As in rabbit colon (10), preliminary experiments with the rat jejunum showed a more prominent vanadate effect when the anion was applied to the serosal instead of the mucosal side of the epithelium.

Influx across the brush border membrane. In the second series of experiments, influx (Jmc) of alanine across the mucosal surface of the rat jejunum was measured as previously described (28, 29). The jejunum was mounted in a chamber which allowed an area of 0.6 cm^2 to be incubated with both its mucosal and its serosal surfaces exposed to control and test

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media. The intestine was preincubated for 30 min in phosphate buffer containing varying concentrations of vanadate. After preincubation, the mucosal surface was exposed for 60 sec to a test solution containing the buffer, [^{14}C]alanine and [^{3}H]inulin. Jejunum was immediately cut and extracted in 0.1 N HNO_3. The extract was counted in a two-channel liquid scintillation counter. Influx was calculated from the ^{14}C counts in the tissue after making a correction for the inulin space (28).

Alanine efflux across the mucosal border. Experiments of the third series of studies were designed to estimate alanine efflux and were similar to those described earlier (30). Briefly the intestine was loaded with ^{14}C-labeled alanine and the appearance of radioactivity in unlabeled mucosal solution was measured. The cells were loaded by incubating a segment of jejunum for 60 min in a solution containing labeled alanine. At the end of the loading period, the tissue was dipped in an isoosmotic mannitol solution to remove excess radioactivity and mounted as a flat sheet in a Lucite chamber. Unlabeled solution (2 ml) was placed on the mucosal side and left for 1 min then replaced by fresh solution. Washout of radioactivity was followed for ten 1-min periods, following which the tissue was removed from the chamber and the mucosa was separated from the muscle layer with a glass slide. The mucosa was extracted for 4 hr in 0.1 N HNO_3 and the radioactivity in the tissue extract was determined to estimate radioactivity remaining in the tissue at the end of the washout periods. Calculation of alanine efflux and of cell alanine concentration was done as described earlier (30). The experimental design of these studies was such that in each experiment, alanine efflux was compared in paired jejunal tissues of the same animal, so that one of the tissues was preincubated in the presence of 10^{-4} M vanadate and 2.5 mM alanine and the other in 10^{-2} M vanadate and 15 mM alanine. Preliminary experiments showed that such incubation provided similar steady-state cell alanine concentrations in the presence of the two tested vanadate concentrations. Efflux also, was compared between tissues pretreated with 10^{-2} M vanadate and 15 mM alanine and paired tissues preincubated with normal buffer solutions containing 2 mM alanine. The cell alanine concentration in these two conditions were again comparable.

Enzyme assays. In the fourth series of experiments, ATPase enzymes and cyclic AMP were measured in rat jejunal mucosa that have been preincubation with vanadate. Briefly, mucosal tissues from jejunal segments were obtained by scraping with a glass slide. The tissues were incubated in a flask for 1 hr at 37°C in a phosphate buffer solution containing varying amounts of vanadate. At the end of preincubation, mucosal homogenates and basolateral membrane fractions (BLM) were obtained as described by Hoyumpa *et al.* (31). ($Na^+ + K^+$)-ATPase was measured in the BLM fractions by the method of Sha'afi *et al.* (32) and cyclic AMP in mucosal homogenates was determined by a radioimmunoassay method using commercial kit (Rianen, Research Products–Dupont, Boston, MA).

Chemicals and radioisotopes. All chemicals used were of analytical grade. Sodium vanadate (Ortho) was obtained from Pfaltz & Bauer, Inc. (Stamford, CT), and L-alanine was from Sigma Chemical Co. (St. Louis, MO). SQ 22,536 was generously supplied by Squibb & Sons, Inc. (Princeton, NJ). [^{14}C]L-alanine and [^{3}H]inulin were from NEN (Research Products–DuPont).

Statistical analyses. The data are expressed as means ± SE. Comparisons between various experimental and control groups were done using Student's two-tailed t test for unpaired data except for the efflux studies shown below where paired comparisons were made.

Results. The results of the first series of experiments are summarized in Table I. Since in previous studies, alanine was very minimally metabolized *in vitro* (30), we studied the effect of vanadate on its unidirectional fluxes. The mucosal-to-serosal flux of alanine was inhibited by $10^{-4}M$ vanadate concentration and the inhibition increased with higher concentrations (10^{-3} and $10^{-2}M$). The opposing serosal-to-mucosal flux of alanine was affected by vanadate only at the highest concentration used ($10^{-2}M$), which caused an increase in the Jsm flux. The changes in the Jms and Jsm fluxes are reflected in the calculations of the net transmural fluxes (Jnet), where Jnet = Jms − Jsm. As shown in Table I, Jnet was significantly reduced at the 10^{-4}, 10^{-3}, and 10^{-2} M

TABLE I. EFFECT OF VANADATE ON UNIDIRECTIONAL FLUXES OF ALANINE AND ELECTRICAL CHARACTERISTICS IN RAT JEJUNUM

Vanadate concentration (M)	0	10^{-8}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Jms (μmole/hr/cm^2)	0.48 ± 0.02	0.44 ± 0.03	0.39 ± 0.03	0.38 ± 0.03	0.26 ± 0.03*	0.13 ± 0.02*	0.10 ± 0.01*
Jsm (μmole/hr/cm^2)	0.06 ± 0.003	0.07 ± 0.004	0.07 ± 0.002	0.07 ± 0.002	0.06 ± 0.004	0.07 ± 0.002	0.09 ± 0.002*
Jnet (μmole/hr/cm^2)	0.42 ± 0.008	0.37 ± 0.016	0.32 ± 0.015	0.31 ± 0.016	0.20 ± 0.17*	0.06 ± 0.008*	0.01 ± 0.001*
Isc (μamp/cm^2)	105 ± 6	107 ± 5	98 ± 5	93 ± 6	73 ± 3	11 ± 1.4*	2 ± 0.8*
Tissue conductance (G, msec/cm^2)	21 ± 3	24 ± 3	26 ± 4	26 ± 5	29 ± 6	29 ± 6	34 ± 6*

Note. Values are means ± SE of studies in eight animals. Alanine concentration in mucosal and serosal media was 2.5 mM; vanadate was added only to the serosal medium. The electrical short-circuit measurements (Isc) represent the average values observed during the 30th and 40th min of incubation. G is the ratio of the short-circuit current to open-circuit transepithelial electrical potential difference.
* $P < 0.01$ as compared to controls in the absence of vanadate.

vanadate concentrations. The Jms and Jnet changes of alanine corresponded well with the changes of the short-circuit current (Isc) that were simultaneously measured on the same tissues. The short-circuit current was significantly reduced at 10^{-4} M vanadate and it almost disappeared at the 10^{-3} and the 10^{-2} M concentrations. The decrease in current was not associated with any significant change in tissue conductance except at the 10^{-2} M concentration where an increase in conductance was observed.

The second series of experiments (Table II) showed evidence for the presence of a dual action of vanadate on alanine influx across the brush border membrane of the rat jejunum. As shown in the table, there was an enhancement in the influx of alanine in the presence of 10^{-4} M of vanadate and a reduction in the influx of the amino acid at the 10^{-3} and 10^{-2} M vanadate concentrations. The decrease in alanine influx at the latter concentrations corresponded well with the observed reduction of the short-circuit current measured in the transmural flux studies above (Table 1). The increase in alanine influx at the 10^{-4} M concentration of vanadate was eliminated when the tissue was preincubated in the presence of 5 mM SQ 22,536, a reported adenylate cyclase inhibitor (33). In control experiments SQ 22,536 had no significant reduction in alanine influx when it was added without vanadate to the preincubation solutions.

While the higher concentrations of vanadate produced uniform inhibition of the influx and of the transmural fluxes of alanine, a difference was noted when the effects of the 10^{-4} M concentration were compared. As noted above,

TABLE II. EFFECT OF VANADATE ON ALANINE INFLUX ACROSS THE MUCOSAL BORDER OF THE RAT JEJUNUM

Vanadate concentration (M)	Influx (μmole/hr/cm^2)						
	0	10^{-8}	10^{-6}	10^{-5}	10^{-4}	10^{-3}	10^{-2}
Buffer solution	1.05 ± 0.06	0.89 ± 0.07	0.93 ± 0.07	1.16 ± 0.08	1.66 ± 0.13 $P < 0.01$	0.68 ± 0.05 $P < 0.01$	0.62 ± 0.04 $P < 0.01$
Buffer solution + 0.5 mM SQ 22536	0.89 ± 0.07				0.69 ± 0.05 $P < 0.05$ ($P < 0.001$)		

Note. Values are means ± SE of studies in eight rats (32 determinations). Alanine concentration in test solutions was 2.5 mM. P values are comparisons with the controls in the absence of vanadate. The P value in parentheses is a comparison between the influx values in the presence of 10^{-4} M vanadate but with or without SQ 22,536.

this concentration caused inhibition of the transmural fluxes and stimulation of the influx. Such a difference can be brought out only if we assume an increase in the efflux of alanine across the brush-border membrane. To test this, the third series of experiments were done (Table III) in which two sets of paired studies were compared. In one set tissues preincubated in 10^{-4} or 10^{-2} *M* vanadate were compared. The mean cell alanine concentration for each group of eight tissues was 23.6 ± 2.5 m*M* for the 10^{-4} *M* group and 22.4 ± 2.0 for the 10^{-2} *M* group. In a second set of experiments tissues preincubated in 10^{-2} *M* vanadate solution were compared with tissues that were preincubated in normal buffer solution. Cell alanine concentrations in these two groups of tissues were respectively 18.4 ± 2.6 and 17.4 ± 3.0 m*M*. To consider the variations in the number of cells present in the chamber, efflux was calculated (30) as microequivalents per hour per milligram dry weight of mucosa. Alanine efflux, under the conditions described, was greater ($P < 0.001$) from tissues preincubated with 10^{-4} *M* than from tissues preincubated in 10^{-2} *M* or from controls.

The results of the enzyme studies (fourth series) are shown in Table IV and they provide additional clarification about the action of vanadate on alanine transport. There were no changes in the activities of the ATPase enzymes at vanadate concentrations that are lower than 10^{-4} *M*, but at 10^{-3} and 10^{-2} *M* reductions in ($Mg^{2+} + Na^+ + K^+$)-ATPase and ($Na^+ + K^+$)-ATPase were observed. The 10^{-4} *M* vanadate concentration caused a minimal but statistically significant reduction in ($Na^+ + K^+$)-ATPase and an increase in cyclic AMP in mucosal homogenates.

Discussion. Exposure of the intestinal epithelium to vanadate in concentrations of 10^{-4} to 10^{-2} *M* produced a reduction in mucosal-to-serosal and net fluxes of alanine, as well as a decrease in Isc. The inhibitory changes observed became more prominent with rising vanadate concentrations and net alanine transport was only minimal at 10^{-3} and 10^{-2} *M* concentrations. The changes in the transmural fluxes of alanine and in the Isc are similar to the changes that are produced by ouabain on intestinal amino acid transport (23) and they represent an inhibitory effect of vanadate on the ($Na^+ + K^+$)-ATPase of the intestinal cell. While it may be possible that the inhibitory effect of vanadate in homogenates may not reflect the activity of ($Na^+ + K^+$)-ATPase in the intact tissues, there was, however, a decrease in the short-circuit current which provides another evidence for the inhibition of the enzyme in the intact tissues after exposure to the higher vanadate concentrations. The inhibition of this enzyme was demonstrated in the studies shown in Table IV, and may be similar to the action of vanadate on the sodium pump of other tissues (1, 2, 8, 9).

TABLE III. EFFECT OF VANADATE ON ALANINE EFFLUX (μmole/hr/mg dry wt) ACROSS THE MUCOSAL BORDER OF RAT JEJUNUM

(a) 10^{-4} *M*	(b) 10^{-2} *M*	Ratio (a/b)	(c) 10^{-2} *M*	(d) control	Ratio (c/d)
0.30	0.18	1.67	0.20	0.17	1.18
0.28	0.23	1.22	0.19	0.16	1.19
0.31	0.21	1.48	0.24	0.14	1.71
0.28	0.22	1.27	0.19	0.14	1.06
0.27	0.19	1.19	0.23	0.20	1.15
0.23	0.21	1.10	0.22	0.12	1.83
0.26	0.20	1.30	0.23	0.20	1.15
0.28	0.22	1.27	0.22	0.16	1.38
Mean ± SEM		1.35 ± 0.18			1.33 ± 0.29
P		< 0.001			< 0.01

Note. Values represent alanine efflux rates in two sets of eight paired experiments with (a) and (b) comparing tissues preincubated respectively in 10^{-4} or 10^{-2} *M* vanadate concentrations and (c) and (d) comparing tissues preincubated in 10^{-2} *M* vanadate and buffer solution without vanadate. *P* values were determined using Student's *t* test for paired observations.

TABLE IV. EFFECT OF VANADATE ON BASOLATERAL MEMBRANE ATPases AND ON MUCOSAL CYCLIC AMP

Vanadate concentration (M)	($Mg^{2+} + Na^+ + K^+$) -ATPase	(Ouabain-insensitive ATPase) (μmole Pi/mg protein/hr)	($Na^+ + K^+$)-ATPase	cAMP (pmole/mg protein)
0	456 ± 32	372 ± 27	85 ± 8	11.1 ± 0.8
10^{-8}	442 ± 29	356 ± 21	86 ± 7	10.3 ± 0.6
10^{-6}	440 ± 27	349 ± 25	90 ± 7	10.6 ± 0.6
10^{-5}	405 ± 20	319 ± 19	82 ± 4	13.7 ± 0.8
10^{-4}	379 ± 23	309 ± 18	70 ± 3*	18.0 ± 0.9*
10^{-3}	317 ± 22**	285 ± 13	32 ± 2***	11.9 ± 0.7
10^{-2}	261 ± 19***	249 ± 8	11 ± 1***	10.5 ± 0.6

Note. ATPase values are the means ± SEM of seven determinations. The cyclic AMP determinations are the means ± SEM of five determinations. Cyclic AMP was significantly increased ($P < 0.05$) at 10^{-4} M vanadate as compared to controls.

* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ as compared to controls that were preincubated without vanadate.

The action of vanadate on the Na-pump does not, however, explain the observed increase in the influx of alanine at the 10^{-4} M vanadate concentration. We believe that enhancement of the influx at this concentration is caused by stimulation of the adenylate cyclase system. The studies in which SQ 22,536 was used (Table II) and the enzyme assay tests (Table IV) support this proposition. The inhibitory action of SQ 22,536 on the vanadate-induced enhancement of alanine influx provided indirect proof for the involvement of adenylate cyclase in the stimulatory action of vanadate at this concentration. This agent, at the 5 mM concentration used, acted as an inhibitor of adenylate cyclase (33) and it prevented the vanadate-stimulated enhancement of alanine influx which occurred when the intestine was preincubated with vanadate alone. Also, the observed increase in mucosal cyclic AMP at the 10^{-4} M concentration supports this possibility. In studies of rat jejunal segments (24) and in brush-border membrane preparations (25) cyclic AMP has been reported to cause a similar degree of stimulation of amino acid transport and vanadate in some systems has been known to increase cyclic AMP (17–21). What is unclear now, however, is the reason for the limited stimulation of cyclic AMP by only a low concentration of vanadate (10^{-4} M). The higher vanadate concentrations tested produced no elevations of the cyclic AMP content of the mucosal homogenates but they actually decreased ($Na^+ + K^+$)-ATPase and inhibited the influx of alanine by reducing the Na^+-gradient across the brush-border membrane.

Despite the fact that a 10^{-4} M concentration of vanadate caused inhibition in the mucosal-to-serosal flux of alanine, the same vanadate concentration increased the influx of the amino acid across the mucosal barrier of the intestinal epithelium. These two observations may seem contradictory but are explainable by assuming that the 10^{-4} M concentration of vanadate causes an increase in the permeability of the mucosal membrane. As shown in Table IV the efflux of alanine was higher in jejunal tissues that were preincubated in a 10^{-4} M concentration of vanadate than in tissues preincubated in 10^{-2} M. The increase in efflux at the 10^{-4} M concentration reconciles the findings of the increase in influx and the decrease in transmural flux at the 10^{-4} M concentration. Thus, the increase in both the entry and the exit of alanine across the mucosal membrane of the intestinal cell leads to no changes or even to a decrease in the transmural unidirectional and net fluxes of alanine.

In conclusion, the studies presented show the presence of at least two effects of vanadate on the amino acid transport mechanism, a stimulatory effect occurring at low vanadate concentrations and causing an increased entry and exist of amino acids into the mucosal border of the intestinal cell, and an inhibitory effect at higher vanadate concentrations causing inhibition of net and transmural absorptive

fluxes of the amino acids across the intestinal epithelium. The results presented provide evidence to suggest that the stimulatory effects are resulting from an increased adenylate cyclase activity and the inhibitory effects from a decreased activity of Na-K-ATPase. While it is known that vanadate has varied actions on different tissues, to our knowledge this is the first report of the existence of a dual action of vanadate on the same tissue. Other studies are needed, however, to further characterize these effects and to determine whether they have a regulatory influence on other transport functions of the intestinal epithelium.

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