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Effects of vanadate on water and electrolyte transport in rat jejunum

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The effect of vanadate (orthovanadate, VO_4^-) on water and ion transport was studied in rat jejunum. Water transport was tested by single-pass perfusion in vivo and ion fluxes by the Ussing-chamber technique in vitro. The results suggest that vanadate has two actions on ion and water transport: (1) At low concentrations (10^{-4} M) it causes Cl^-, Na^+ and water secretion by stimulation of adenylate cyclase; (2) At higher concentrations (10^{-3} and 10^{-2} M) it decreases net absorption of Na^+ and Cl^- by inhibition of $(Na^+ + K^+)$-ATPase.

The pentavalent vanadium ion, vanadate, has been reported to inhibit $(Na^+ + K^+)$-ATPase, and in some tissues, to stimulate adenylate cyclase [1–5]. Since transport across the intestinal epithelium is influenced by both enzymes, we studied the effect of vanadate on net water transport in the in vivo rat intestine and on the transmural fluxes of Na^+ and Cl^- across the isolated rat jejunum. We report evidence to suggest that vanadate depending on concentration, may have a dual action on both adenylate cyclase and $(Na^+ + K^+)$-ATPase of rat jejunum.

Male Sprague-Dawley rats weighing 250–300 g were used and studied under in vivo and in vitro conditions. In the in vivo studies, the rats were anesthetized with intraperitoneal sodium pentobarbital injection (45 mg/kg) and jejunal segments (20–25 cm) of their intestines were tested in situ using a single-pass perfusion technique as that previously reported [6]. Briefly, the segments were perfused (0.5 ml/min) with isotonic saline solution containing ^{14}C-labeled polyethylene glycol (^{14}C-PEG, NEN Research Products-Dupont, Boston, MA), a nonabsorbable indicator. Sodium vanadate was added to the perfusate and effluent samples were collected during a 100 min period of perfusion. The perfusates in control animals contained mannitol added in isoosmotic amounts as the vanadate of the experimental group. The sample collected during the first 40 min of equilibration was discarded and the sample collected during the next 60 min tested for its ^{14}C-PEG content. Absorption per dry weight of the perfused segments was calculated as described earlier [7].

Unidirectional transmural fluxes of $^{22}Na^+$ and $^{36}Cl^-$ (NEN, Research Products-Dupont, Boston, MA) were studied by a method similar to that described by Munck [8]. Mucosal-to-serosal (J_{ms}) and serosal-to-mucosal (J_{sm}) fluxes were determined simultaneously in four segments of jejunum. Two tissues were bathed by Krebs-phosphate buffer (controls) and the other two exposed to vanadate added to the buffer bathing their serosal side. As in rabbit colon [9], a more prominent vanadate effect was observed when the anion was applied to the serosal instead of the mucosal side of the intestine.

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TABLE I

EFFECT OF VANADATE ON WATER ABSORPTION IN IN VIVO RAT JEJUNUM

Values are means ± S.E. of observations in eight rats. Absorption is presented per dry weight of perfused intestinal segments. Negative values indicate net secretion, positive, absorption. In control conditions mannitol was added in isoosmotic quantities as the corresponding vanadate concentrations.

Vanadate concn. (M)	Water absorption (μl/h per g)	
	Control	Vanadate
0	3.8 ± 0.3	3.9 ± 0.4
10^{-8}	4.0 ± 0.4	3.8 ± 0.5
10^{-6}	3.8 ± 0.5	3.6 ± 0.4
10^{-4}	3.9 ± 0.6	3.7 ± 0.3
10^{-3}	3.6 ± 0.4	−3.1 ± 0.6 [a]
10^{-2}	3.4 ± 0.4 [b]	−5.0 ± 0.6 [a]

[a] $P < 0.001$ as compared to control absorption in the absence of vanadate but in the presence of an isoosmolar amount of mannitol.

[b] $P < 0.05$ as compared to control value in the absence of mannitol (Student's t-test).

In another series of experiments, $(Na^+ + K^+)$-ATPase and cyclic AMP were measured in jejunal segments that were preincubated with vanadate for 1 h. $(Na^+ + K^+)$-ATPase was measured by the method of Sha'afi et al. [10] and on basolateral-membrane fractions prepared as described by Hoyumpa et al. [11]. Cyclic AMP in mucosal homogenates was measured by a radioimmunoassay method using a commercial kit (Rianen, Research Products-Dupont, Boston, MA).

The effect of vanadate on water absorption is shown in Table I. The addition of mannitol (0–20 mM) to the perfusates in the control conditions caused no significant changes in water absorption except at the highest concentration where net water absorption was only minimally reduced by the presence of 20 mM of mannitol in the isotonic saline solution (equivalent to the 10^{-2} M of vanadate). When vanadate in concentrations of 10^{-8} to 10^{-4} M were added they caused no changes in water absorption. However, at the higher concentrations of 10^{-3} and 10^{-2} M, significant secretion of water occurred. The secretion was remarkable and not accountable by the osmotic presence of vanadate in the perfusate. It is possible to explain the observed secretion of water to be due to inhibition of $(Na^+ + K^+)$-ATPase which could stop the absorptive flow of water into the epithelium and the normally existing secretory flow becomes evident. Another explanation is that the increase in secretion of water in vivo is of a similar nature as the secretion of Cl^- and Na^+ that was observed in the in vitro experiments shown in Table II. While it is difficult to compare the in vivo and the in vitro conditions of the present experiments, the secretion of water in vivo and of the ions in vitro raises the possibility that the effect of vanadate on these two transport processes may be of a similar nature. The secretion of water in vivo occurred, however, at a higher vanadate concentrations (10^{-3}–10^{-2} M) than that (10^{-4} M) which caused the secretion of Cl^- and Na^+ in vitro. This discrepancy could however, be related to differences in absorption and in intracellular distribution of vanadate between these two conditions. Another possibility is that it is caused by differences in the application of vanadate to either the luminal or serosal sides of the epithelium. In preliminary experiments we injected vanadate (12 mg/kg) intraperitoneally before the perfusion studies and despite the large dose used (75% of the lethal dose), vanadate caused no changes in water absorption (data not shown). We felt therefore, that parenteral vanadate could not be used to study the intestine since as reported by Sharma et al. [12] intraperitoneal administration of the oxyanion leads to its accumulation in the liver and spleen with only minimal amounts reaching the intestines. It is furthermore, unclear now how effective is vanadate absorption when it is perfused luminally and whether intracellular vanadate is actually high in the in vivo conditions of the above experiments. As suggested by Cantley and Aisen [13], the physiological activity of vanadate is influenced by its intracellular concentration. We assume a low cell vanadate concentration is obtained when the anion is perfused in vivo even when the perfusates contain the high concentrations of 10^{-3} and 10^{-2} M. Such an assumption is considered reasonable because vanadate transport into the intestine occurs by a passive energy-independent process [14] and the cell vanadate concentration cannot be too different between the in vivo and the in vitro conditions of the present experiments.

The results of the transmural fluxes are sum-

TABLE II

EFFECT OF VANADATE ON ION FLUXES, I_{sc}, CONDUCTANCE AND RESIDUAL FLUXES ACROSS ISOLATED RAT JEJUNUM

Values are means ± S.E. of observations in eight animals. I_{sc} (short-circuit current) was measured in μA and converted to μequiv./h per cm^2 by multiplication by 3600 and division by surface area times faraday's constant. Electrical conductance was determined by dividing I_{sc} by the open circuit PD that was measured intermittently during the flux studies. J_r was calculated by the formula $J_r = [I_{sc} - (J_{net}(Na) - J_{net}(Cl))]$.

Vanadate concn. (M)	Na fluxes (μequiv./h/cm^2)			Cl fluxes (μequiv./h/cm^2)			I_{sc} (μequiv./h/cm^2)	G (mmho/cm^2)	J_r (μequiv./h/cm^2)
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}			
0	7.1 ± 0.3	6.1 ± 0.2	1.7 ± 0.1	8.4 ± 0.4	8.3 ± 0.3	0.1 ± 0.01	3.2 ± 0.2	22	1.5 ± 0.1
10^{-8}	7.7 ± 0.4	6.1 ± 0.2	1.6 ± 0.1	8.6 ± 0.4	8.4 ± 0.4	0.2 ± 0.01	3.2 ± 0.2	24	1.8 ± 0.1
10^{-6}	7.2 ± 0.4	6.1 ± 0.4	1.1 ± 0.1	8.2 ± 0.5	8.4 ± 0.4	−0.2 ± 0.01	2.7 ± 0.2	25	1.4 ± 0.1
10^{-4}	5.0 ± 0.4 [a]	6.7 ± 0.3	−1.7 ± 0.1 [a]	5.9 ± 0.3 [a]	9.0 ± 0.5 [a]	−3.0 ± 0.2 [a]	2.8 ± 0.2	27	1.5 ± 0.1
10^{-3}	6.2 ± 0.3	6.2 ± 0.3	0.1 ± 0.01 [a]	6.4 ± 0.4 [b]	6.3 ± 0.3 [b]	0.1 ± 0.01	1.0 ± 0.1 [b]	27	1.1 ± 0.04
10^{-2}	6.6 ± 0.4	6.6 ± 0.4	−0.1 ± 0.01 [a]	6.6 ± 0.3 [b]	6.7 ± 0.4	−0.1 ± 0.01	0.1 ± 0.01 [a]	37	0.1 ± 0.01 [a]

[a] $P < 0.001$, [b] $P < 0.01$, as compared to control measurements in the absence of vanadate (Student's t-test).

marized in Table II. The presence of 10^{-8} and 10^{-6} M vanadate concentrations caused no changes in the transmural fluxes of Na^+ or Cl^- and no changes in the transmural short-circuit current (I_{sc}). The mucosal-to-serosal fluxes of Na^+ and Cl^- were however, reduced by 10^{-4} M of vanadate, while the J_{sm} of chloride unlike that of Na^+ was significantly increased at this concentration. The changes in the unidirectional fluxes of Na^+ and Cl^- lead to significant net secretion of Cl^- and Na^+. These changes are similar to the ion transport changes that are reported to happen after the exposure of the intestine to cyclic AMP [15]. At the higher concentrations of 10^{-3} and 10^{-2} M, vanadate produced significant reductions in the net flux of sodium and in the short-circuit current (I_{sc}). The decrease in current at 10^{-3} M was not associated with any change in tissue conductance, while at the 10^{-2} M the tissue conductance was significantly increased. The decrease in transmural fluxes and in I_{sc} at the 10^{-3} and 10^{-2} M concentrations are probably the result of an inhibitory effect of vanadate on the Na^+ pump.

The enzyme studies in Table III provide an explanation for the observed differences between the low and high concentrations of vanadate on water and ion transport. At the 10^{-4} M concentration there is an increase in cyclic AMP and

TABLE III

EFFECT OF PREINCUBATION OF RAT JEJUNUM IN VANADATE ON BASOLATERAL MEMBRANE ATPASES AND ON MUCOSAL CYCLIC AMP

ATPase activity values are means ± SE of six observations presented as μmol P_i/mg protein. The cyclic AMP values represent determinations in a single representative experiment. An increase in cyclic AMP at 10^{-6} and 10^{-4} M vanadate concentrations was also observed in two other similar experiments.

Vanadate concn. (M)	$(Mg^{2+} + Na^+ + K^+)$-ATPase	Ouabain insensitive ATPase	$(Na^+ + K^+)$-ATPase	cAMP (pmol/mg protein)
0	462 ± 23	370 ± 23	92 ± 7	10.5
10^{-8}	449 ± 24	362 ± 18	87 ± 6	10.3
10^{-6}	438 ± 26	351 ± 20	87 ± 6	16.4
10^{-4}	358 ± 28	305 ± 28	53 ± 4 [a]	18.6
10^{-2}	266 ± 21 [b]	240 ± 17 [b]	26 ± 2 [b]	10.8

[a] $P < 0.05$, [b] $P < 0.01$ as compared to controls in the absence of vanadate (Student's t-test).

a minimal decrease in $(Na^+ + K^+)$-ATPase. The vanadate-stimulated increase in cyclic AMP is very likely responsible for the observed secretion of Cl^- and Na^+ by vanadate. The stimulation of cyclic AMP is however, only limited to the 10^{-4} M concentration. We have no explanation about why cyclic AMP is stimulated at low but not at high concentration of vanadate. Normally, cyclic AMP stimulates active Cl^- secretion which is associated with passive transepithelial secretion of Na^+ and with an increase in transmural potential difference and in short-circuit current [15]. In the present experiments the short-circuit current did not rise, despite Cl^- and Na^+ secretion. This suggest that either coupling of Na-Cl happens during secretion or that the associated inhibition of $(Na^+ + K^+)$-ATPase at the 10^{-4} M concentration may have countered some of the effect of cyclic AMP and prevented the expected rise in the short-circuit current. At the higher vanadate concentration of 10^{-2} M, the $(Na^+ + K^+)$-ATPase was markedly inhibited and the observed changes in ion transport were undoubtedly caused by this inhibition.

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. Depending on concentration, vanadate appears to exert its effect on ion transport through stimulation of adenylate cyclase and inhibition of $(Na^+ + K^+)$-ATPase.

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