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VANADIUM ION STIMULATION OF CHLORIDE SECRETION BY RABBIT COLONIC EPITHELIUM

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Ionized forms of vanadium are known to exert diverse biological activities. Of particular interest is the inhibitory action of the vanadium ion on ($Na^+ + K^+$)-ATPase. This report describes another action of the vanadium ion on the rabbit colonic epithelium. Micromolar quantities of vanadate, applied to the serosal side of the isolated rabbit colonic epithelium, result in a stimulation of electrogenic chloride secretion by this epithelium. Sodium transport is unaffected by the vanadium ion in the concentrations used in this study. It is proposed that the vanadyl ion activates adenylate cyclase and thereby initiates subsequent secretory events.

Introduction

It is evident from recent reports concerning the actions of vanadium, that ionized forms of this element exert diverse biological activities in a variety of cellular systems. By far the most cited action of the vanadium ion has been its inhibitory action on ($Na^+ + K^+$)-ATPase in mammals [1,2], reptiles [3], amphibians [4,5] and fungi [6]. Other actions include the inhibition of Ca^{2+}-ATPase in mammalian red blood cells [7,8] and mammalian cardiac tissue [8], and H^+-stimulated ATPase in turtle bladder [9]. In addition, it is known to inhibit several phosphatases [4,10,11] and kinases [1]. In contrast to its inhibitory actions, the vanadium ion stimulates adenylate cyclase [1,7,12,13] and cAMP activity within rat fat cells [13] and cardiac tissue [12]. Depending on species, vanadium is also reported to have both positive or negative inotropic effects on cardiac cells [7], and in addition is known to be a powerful vasoconstricting agent [14].

This report describes yet another action of vanadium which was demonstrated in rabbit colonic epithelium. Despite its reported ouabain-like action in a number of tissues, vanadium had no effect on rabbit colonic sodium transport. Rather, the major effect of the vanadium ion in this tissue was a stimulation of chloride secretion.

Materials and Methods

Descending colonic tissues from male New Zealand White rabbits (2–3 kg) were partially stripped of serosal layers [15] and mounted as flat sheets in standard Ussing chambers. Both sides of the tissue were bathed with identical buffer solutions circulated with 95% O_2/5% CO_2 gas lift pumps and maintained at 37°C. The buffer solution contained the following, in mM: 140 Na^+; 5.4 K^+; 1.2 Ca^{2+}; 1.2 Mg^{2+}; 123 Cl^-; 21 HCO_3^-; 2.4 HPO_4^{2-}; 0.6 $H_2PO_4^-$ and 10 glucose.

Tissues were short-circuited with automatic voltage clamps (DVC: 100, WP Instruments), which compensated for the solution resistance.

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Tissue conductance (G_T, mS/cm^2) was calculated as the ratio of the short-circuit current (I_{sc}, μA/cm^2) to the open-circuit transepithelial electrical potential difference (mV).

Unidirectional tracer fluxes ($J_{m\rightarrow s}^{Na^+/Cl^-}$, $J_{s\rightarrow m}^{Na^+/Cl^-}$) of sodium and chloride were determined by adding either $^{22}Na^+$ or $^{36}Cl^-$ to one bathing solution and measuring its appearance in the opposite solution. Flux measurements were made every 20 min and the mean of three or four flux determinations is presented for each control and experimental period. Net fluxes ($J_{net}^{Na^+/Cl^-}$) were calculated as the difference between the two unidirectional fluxes from adjacent pieces of tissue of similar conductance.

Sodium orthovanadate and ouabain were obtained from Sigma Chemical Co., while sodium metavanadate was obtained from Calbiochem. Furosemide and amiloride were generously provided by Hoechst-Roussel Pharmaceuticals and Merck, Sharpe & Dome, respectively. All other chemicals were reagent grade and were obtained from local suppliers.

Freshly made vanadate solutions contain polynuclear vanadium complexes ($V_3O_9^{3-}$, $V_4O_{10}^{4-}$ and decavanadates $V_{10}O_{28}H^{5-}$) which slowly dissociate and it is recommended that these solutions be boiled or left standing for several days. The solutions used in these experiments were prepared immediately before their addition; however, solutions left standing for 7 days or longer at 4°C produced similar responses in colonic tissues. Under the experimental conditions used, the vanadate ion (V in the +5 oxidation state) was presumed to predominate. In addition, no difference in the potency of action was observed between sodium orthovanadate (Na_3VO_4) and sodium metavanadate ($NaVO_3$).

Results are presented as the mean value plus or minus one standard error ($\bar{x} \pm$ S.E.) based upon the number (n) of tissues or tissue pairs studied. Student's t-test was used to evaluate the differences between means of paired tissues. Comparisons of more than two treatment means were made using a one-way analysis of variance and Duncan's Multiple Range test [16]. Unless otherwise noted, differences are considered significant if $P \leqslant 0.05$.

Results

The effects of serosal vanadate on the transport and electrical characteristics of the isolated rabbit colon are presented in Table I. During control periods, net absorptive fluxes (m → s) of both Na^+ and Cl^- were apparent (see also Tables II and III). $1 \cdot 10^{-4}$ M serosal vanadate promoted a 2-fold increase in I_{sc} and reversed the direction of net chloride transport from absorption to secretion. This change in J_{net}^{Cl} was attributable primarily to an increase in J_{sm}^{Cl}. Serosal addition of vanadate was without effect on the sodium transport prop-

TABLE I

UNIDIRECTIONAL AND NET FLUXES OF Cl^- AND Na^+ AND ELECTRICAL CHARACTERISTICS OF RABBIT COLONIC MUCOSA, BEFORE AND AFTER SEROSAL VANADATE ADDITION

Results are given as means ± S.E. n = number of tissues; N = number of animals; n.s. not significant. J in μequiv./cm^2 per h.

Condition	J_{sm}^{Cl}	J_{ms}^{Cl}	J_{net}^{Cl}	J_{sm}^{Na}	J_{ms}^{Na}	J_{net}^{Na}	I_{sc} (μA/cm^2)	G_T (mS/cm^2)
Control	3.06 ±0.30	4.50 ±0.34	1.44 ±0.76	0.93 ±0.16	1.85 ±0.31	0.91 ±0.20	0.74 ±0.09	3.11 ±0.21
10^{-4} M vanadate (serosal)	4.72 ±0.31	3.67 ±0.38	−1.05 ±0.32	1.34 ±0.20	2.44 ±0.41	1.10 ±0.28	1.68 ±0.18	3.58 ±0.22
n	18	18	18	13	13	13	44	44
N	11	11	11	5	5	5	12	12
P	0.001	n.s.	0.001	0.05	0.05	n.s.	0.001	0.01

TABLE II

UNIDIRECTIONAL AND NET FLUXES OF Cl^- AND Na^+ AND ELECTRICAL CHARACTERISTICS OF RABBIT COLONIC MUCOSA, BEFORE AND AFTER MUCOSAL VANADATE ADDITION

Results are given as means ± S.E. n = number of tissues. N = number of animals. Experimental values are not significantly different from controls. J in μequiv./cm² per h.

Condition	J_{sm}^{Cl}	J_{ms}^{Cl}	J_{net}^{Cl}	J_{sm}^{Na}	J_{ms}^{Na}	J_{net}^{Na}	I_{sc} (μA/cm²)	G_T (mS/cm²)
Control	2.16 ±0.35	3.65 ±0.73	1.49 ±0.57	1.18 ±0.30	2.52 ±0.64	1.34 ±0.61	0.86 ±0.21	2.64 ±0.29
10^{-4} M vanadate (mucosal)	2.03 ±0.50	3.84 ±0.85	1.81 ±0.53	1.18 ±0.20	2.37 ±0.47	1.19 ±0.47	1.07 ±0.45	2.66 ±0.21
n	6	6	6	6	6	6	24	24
N	3	3	3	3	3	3	6	6

erties of this tissue. In contrast to the effects of serosal vanadate, no significant difference in the transport or electrical properties of the rabbit colon was observed when $1 \cdot 10^{-4}$ M vanadate was applied to the mucosal solution (Table II).

As shown in Table III, vanadate-induced increases in I_{sc} and chloride transport were readily reversed by $1 \cdot 10^{-4}$ M serosal furosemide. As before, the changes in the direction of net chloride transport and the magnitude of I_{sc} were due to changes in J_{sm}^{Cl}. Furosemide had no effect on the net or unidirectional fluxes of sodium across the rabbit colon. It should also be noted that vanadate-induced increases in I_{sc} were abolished by serosal $1 \cdot 10^{-4}$ M ouabain (from 39.5 ± 2.6 μA/cm² during control period to 2.0 ± 0.43 μA/cm² after ouabain addition), and J_{net}^{Na} was abolished, as was J_{net}^{Cl} secretion.

TABLE III

UNIDIRECTIONAL AND NET FLUXES OF Cl^- AND Na^+ AND ELECTRICAL CHARACTERISTICS OF RABBIT COLONIC MUCOSA, BEFORE AND AFTER SEROSAL VANADATE ADDITION AND AFTER SEROSAL FUROSEMIDE ADDITION

Results are given as means ± S.E. n = number of tissues; N = number of animals. J in μequiv./cm² per h.

Condition	J_{sm}^{Cl}	J_{ms}^{Cl}	J_{net}^{Cl}	J_{sm}^{Na}	J_{ms}^{Na}	J_{net}^{Na}	I_{sc} (μA/cm²)	G_T (mS/cm²)
Control	3.04 ±0.64	3.58 ±0.82	0.54 ±0.41	0.75 ±0.18	1.99 ±0.55	1.25 ±0.40	1.00 ±0.14	3.66 ±0.35
10^{-4} M vanadate (serosal)	3.81 ±0.57	3.28 ±0.59	−0.52 [a] ±0.56	1.55 ±0.47	2.75 ±0.89	1.20 ±0.44	1.97 [a] ±0.23	4.47 ±0.31
10^{-4} M furosemide (serosal)	1.59 [a] ±0.21	3.37 ±0.45	1.79 [a] ±0.48	1.56 ±0.44	3.01 ±0.80	1.44 ±0.43	1.00 [a] ±0.11	4.66 ±0.35
n	5	5	5	5	5	5	20	20
N	2	2	2	2	2	2	4	4

[a] Significantly different from preceding condition ($P < 0.05$).

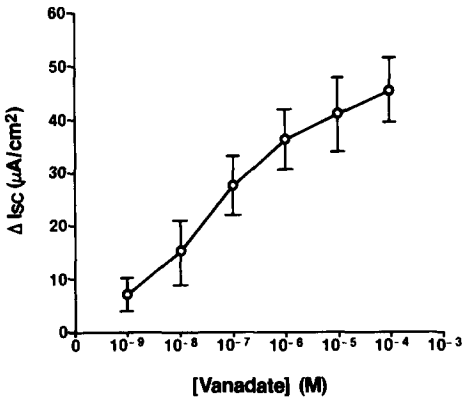

Fig. 1. Dose-dependent increases in I_{sc} (presented as ΔI_{sc}) observed in amiloride-treated colonic mucosa after serosal vanadate addition.

Finally, the vanadate sensitivity of the chloride secretory system was further examined in tissues in which net sodium transport and I_{sc} had been abolished with mucosal amiloride. As shown in Fig. 1, as little as 10^{-9} M serosal vanadate produced a small but measurable increase in I_{sc}. Further increases in serosal vanadate concentrations produced dose-dependent increases in I_{sc}. At vanadate concentrations greater than $1 \cdot 10^{-3}$ M, tissue resistance decreased rapidly, suggesting the integrity of the epithelial barrier was destroyed by high vanadate levels.

Discussion

In recent years the vanadium ion has attracted considerable attention as a potent inhibitor of Na^+,K^+-activated ATPase [1,2]. The ouabain-like activity of nanomolar levels of vanadium has been documented in a variety of cell types from a wide range of organisms [1–6]. The present investigation was initiated to evaluate the possibility of employing vanadate, rather than cardiac glycosides, as a sodium transport inhibitor in the descending colon of the rabbit. However, rather than inhibiting sodium transport, the vanadium ion promoted a reversal in the direction of net chloride transport, from absorption to secretion. This reversal was due primarily to an increase in J_{sm}^{Cl} and, as judged by the increase in I_{sc}, was an electrogenic process. Furthermore, serosal furosemide reversed and serosal ouabain inhibited vanadium ion-induced chloride secretion.

The chloride secretory response produced by vanadium is identical to that produced by cAMP or by secretagogues that elevate intracellular cAMP levels in the rabbit colon (Ref. 17, and unpublished data). According to contemporary models [17], elevated cAMP promotes electrogenic chloride secretion in the tissue by (indirectly) increasing the Cl^- conductance of the apical membrane of the secretory cells. In these cells, intracellular chloride is accumulated against an electrochemical gradient; consequently, increasing the apical membrane Cl^- conductance enhances the outward, passive flow of chloride into the lumen. High intracellular Cl^- activities are maintained by a neutral, Na^+-coupled cotransport system in the basolateral membrane. This pump is driven by the inwardly directed sodium gradient, which is in turn maintained by an ouabain-sensitive ($Na^+ + K^+$)-ATPase also located in the basolateral membrane. While other mechanisms might be suggested, it seems most reasonable to propose that vanadium ion-stimulated Cl^- secretion in the rabbit colon is also mediated by intracellular cAMP. This proposal is consistent with vanadium-induced increases in adenylate cyclase activity [1,2,7,12] and cAMP levels [12] observed in non-intestinal tissues.

The observation that the vanadium ion induces chloride secretion in the rabbit colon while not inhibiting the ($Na^+ + K^+$)-ATPase in this tissue, can be explained by either of two mechanisms: (1) the vanadium ion does not enter the cell, but activates adenylate cyclase by acting upon cell-surface components in a nonspecific manner, or (2) the vanadium ion enters the cell and effects the activation of adenylate cyclase from the cell interior. Since it is generally accepted that the vanadium ion readily penetrates cells and exerts its inhibitory and stimulatory actions intracellularly [4,9,18–21], an intracellular mechanism of action is suggested.

Adenylate cyclase catalyses the intracellular formation of cAMP and it appears that the vanadium ion activates adenylate cyclase in the rabbit colon, thereby initiating the sequence of events leading to chloride secretion. Activation of adenylate cyclase has been reported to be triggered by numerous nonspecific stimuli [22] in addition

to its specific hormonal activation. Millimolar concentrations of fluoride are known to activate this enzyme system in vitro; however, its mechanism of action has not been clearly defined [23]. More recently, molybdate, at millimolar concentrations, has been reported to activate adenylate cyclase in a similar manner as fluoride [24]. The authors suggested that both compounds possibly inhibit a phosphatase, and thereby stabilize the active form of adenylate cyclase. Vanadate stimulation of adenylate cyclase has previously been reported in cardiac tissue from different animals including man [7] and in rat fat cells [13]. In the latter study, the effect of vanadate stimulation of adenylate cyclase was examined in the presence of theophylline. Since the effect of vanadate was unaltered by theophylline, the authors concluded that it was unlikely that vanadate caused an inhibition of phosphodiesterase [13]. In addition, Schmitz et al. [12] report that phosphodiesterase activity in cat cardiac tissue is unaffected by vanadate. Preliminary observations in the rabbit colon would support the conclusion that the vanadium ion does not inhibit phosphodiesterase; however, further study is necessary to validate this conclusion.

Both vanadate (V in the +5 oxidation state) and vanadyl (V in the +4 oxidation state) are known to inhibit a number of phosphatases [10,11], and vanadyl has been known to be more potent than vanadate in inhibiting alkaline phosphatase [11]. This type of inhibition is apparently due to the ability of the vanadium ion, and other transition metal oxyanions such as molybdate and tungstate, to form chelates with the phosphatases at their active site [10,11]. Activation of adenylate cyclase involves GTP and the hydrolysis of GTP to GDP represents the 'turn-off' mechanism [21]. The inhibition of GTPase by the vanadium ion could result in sustaining adenylate cyclase activation and consequently the sequence of events leading to chloride secretion would be maintained.

The 'sidedness' of vanadate action which has been demonstrated previously in other tissues such as toad [4,5] and turtle [3,9] bladder and frog skin [5], is also apparent in rabbit colon. This may simply reflect differences in the permeability of the apical and the basolateral membrane to the vanadium ion. Vanadate apparently enters some cells via disulfonic stilbene-sensitive anion pathways [3,4,19,21] and binds to the phosphorylation site of the $(Na^+ + K^+)$-ATPase from the cytoplasmic side. It has been demonstrated, in red blood cells [8,25,26] and in cardiac cells [8], that while vanadate is the transported ion, once inside the cell vanadate is promptly reduced by enzymic [8] and non-enzymic [26] reactions to vanadyl. Therefore, intracellular vanadium most likely exists exclusively as vanadyl. The lack of vanadium inhibition of rabbit colonic sodium transport which may be inferred to be a lack of inhibition of $(Na^+ + K^+)$-ATPase may be explained by the enzyme's insensitivity to vanadyl. Dubyak and Kleinzeller [19] demonstrated that rat fat cell $(Na^+ + K^+)$-ATPase was insensitive to vanadate and suggested a similar explanation. This is in accordance with in vitro studies which demonstrate the insensitivity of $(Na^+ + K^+)$-ATPase to vanadyl [25,27].

If it is assumed that the vanadium ion-induced chloride secretion, observed in rabbit colon, involves the adenylate cyclase system, the sensitivity of this system is manifest at nanomolar concentrations as demonstrated by the increase in I_{sc} and chloride ion movement in the $s \rightarrow m$ direction. Concentrations of vanadium ion greater than 1 mM were not employed. However, over the range 10^{-9} M to 10^{-3} M no inhibition of sodium transport was observed. In other tissues, particularly renal tissues, nanomolar concentrations of vanadium inhibit $(Na^+ + K^+)$-ATPase [1,2], and hence conjecture has arisen as to whether this element is an endogenous regulator of $(Na^+ + K^+)$-ATPase. Although this concept is novel, the role of vanadium as a broad spectrum $(Na^+ + K^+)$-ATPase regulator is an unlikely one. However, in specific tissues, where intracellular vanadium concentrations reach threshold levels, the determining and limiting factor of inhibitory or stimulatory effects may well be based upon vanadate/vanadyl reactions.

In summary, it has been demonstrated that micromolar quantities of vanadate, applied to the serosal side of the isolated rabbit colon, stimulate the electrogenic secretion of chloride across this epithelium. Sodium absorption is unaffected by any vanadate concentration employed. To account for these observations it is proposed that vanadate is reduced to vanadyl after translocation across the

basolateral membrane. It is suggested that the vanadyl ion increases the activity of adenylate cyclase (and stimulates subsequent secretory events), but is without effect on the activity of the sodium pump.

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