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Vanadium-induced Cl^--secretion in rabbit descending colon is mediated by prostaglandins

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Vanadium in the 4+ (vanadyl-ion) and 5+ (vanadate-ion) oxidation state stimulates furosemide-sensitive electrogenic Cl^- secretion in isolated epithelia of rabbit descending colon. This effect is associated with an increased release of prostaglandin E_2 from the tissue. Inhibitors of phospholipase A_2 or cyclooxygenase abolish both vanadium-induced release of prostaglandin E_2 and Cl^- secretion. Neuronal mechanisms are not likely to be involved, as tetrodotoxin does not affect the vanadate induced Cl^- secretion. Although vanadate is known to inhibit Na^+,K^+-ATPase activity, no inhibition of active Na^+ transport was observed in intact colonic epithelia suggesting a rapid intracellular reduction of vanadate ions to vanadyl ions which have no inhibitory effect on the Na^+,K^+-ATPase. The present findings therefore indicate that vanadate stimulated colonic Cl^- secretion involves intracellular conversion of vanadate to vanadyl and release of prostaglandin E_2.

Introduction

Vanadate, VO_4^{3-}, is known to inhibit the Na^+,K^+-ATPase activity by binding to the ATP hydrolysis site because of its close similarity to phosphate (see Refs. 1 and 2). Since short-circuit current, I_{sc}, in rabbit colon is primarily due to active Na^+ absorption [3], inhibition of Na^+,K^+-ATPase activity is expected to result in a decrease of I_{sc}. Interestingly, after treatment of isolated epithelia of rabbit colon with vanadate no inhibitory effect on transepithelial Na^+ transport was found, rather, vanadate elicits electrogenic Cl^- secretion, in agreement with the earlier report of Hatch et al. [4]. Our study was designed to elucidate the mechanism of the vanadate induced Cl^- secretion.

Parts of this investigation were presented previously in abstract form [5,6].

Methods

Epithelial preparations of rabbit descending colon stripped of the muscle layers were mounted in Ussing-type chambers [3] and short-circuited using an automatic voltage clamp (DVC-1000, W-P Instruments, New Haven, CT, USA). The bathing medium, which contained (mmol/l) 140 Na^+, 124 Cl^-, 21 HCO_3^-, 5.4 K^+, 2.4 HPO_4^{2-}, 0.6 $H_2PO_4^-$, 1.2 Mg^{2+}, 1.2 Ca^{2+}, and 10 glucose was kept at 37°C and gassed with a mixture of 95% O_2 and 5% CO_2, resulting in a pH of 7.4.

Unidirectional transepithelial fluxes of Cl^- and Na^+ were measured by adding ^{36}Cl or ^{22}Na to the solution on one side of the tissue and measuring the steady-state rate of appearance of tracer on the other. Net ion fluxes were obtained from the differences between the unidirectional fluxes in the absorptive and in the secretory direction.

Prostaglandin E_2 was estimated by radioimmunoassay (NEN, Dreieich, Germany). Vanadate or vanadyl dependent release of PGE_2 from the tissue was calculated from the difference between PGE_2 concentrations in the bathing solutions before and 15 min after addition of the vanadium compounds in comparison to controls (no addition of vanadate or vanadyl).

Sodium vanadate was purchased from Sigma Chemie GmbH (Deisenhofen, Germany) and vanadyl sulfate trihydrate from Aldrich Chemie GmbH & Co. KG (Steinheim, Germany). To avoid the formation of vanadate oligomeres the stock solutions of vanadate were boiled prior to use [7].

Statistical significance of differences was calculated by the use of the Student's *t*-test.

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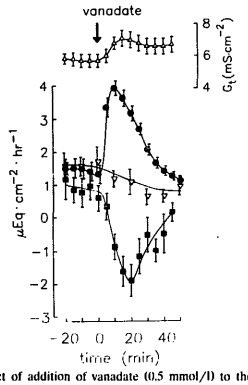

Fig. 1. Effect of addition of vanadate (0.5 mmol/l) to the serosal bathing solution on short-circuit current (I_{sc}, ●——●, $n = 7$), transepithelial net Cl^--flux (J^{Cl}_{net}, ■——■, $n = 7$), net Na^+-flux (J^{Na}_{net}, ▽——▽, $n = 8$), and epithelial conductance (G_t, △——△, $n = 8$) of isolated colonic epithelia. Means ± S.E.

Results and Discussion

Addition of vanadate (0.5 mmol/l) to the solution bathing the serosal side of isolated epithelia of rabbit descending colon caused a rapid increase of I_{sc}, which reached a maximum at 10 min (Fig. 1). Thereafter, I_{sc} declined with a half-life of approx. 20 min. Measurements of unidirectional transepithelial ion fluxes showed that vanadate causes an increase in the serosa-to-mucosa Cl^--flux, resulting in a shift from net Cl^- absorption to net Cl^- secretion. The changes in net Cl^- transport mirror those in I_{sc} in both time course and magnitude. At the same time the electrical conductance of the tissue increased (Fig. 1). Serosal pretreatment of the colonic epithelia with the loop diuretic furosemide abolished the vanadate effect on I_{sc} (Table I). Furosemide inhibits Na^+,K^+,Cl^--cotransport across the basolateral membrane, the cellular uptake step of transepithelial Cl^- transport in secretory epithelia [8,9]. Taken together, these findings show that vanadate elicits furosemide-sensitive electrogenic Cl^- secretion in rabbit colon.

To assess the effect of vanadate on the Na^+,K^+-ATPase activity in colonic epithelia we measured net (or active) transepithelial Na^+ transport. After serosal addition of 0.5 mmol/l vanadate net Na^+ transport was only slightly decreased (Fig. 1). This finding was unexpected since vanadate is known to inhibit Na^+,K^+-ATPase activity. However, it has been shown

TABLE I

Effects of serosal pretreatment with furosemide (0.5 mmol/l) or tetrodotoxin (1 μmol/l), on stimulation of I_{sc} by vanadate (0.5 mmol/l) in isolated colonic epithelia

Furosemide and tetrodotoxin were added 20 min before vanadate. Means ± S.E., n = number of experiments.

Pretreatment	I_{sc} (μequiv. cm^{-2} h^{-1})		
	before addition of vanadate	10 min after addition of vanadate	n
None	0.7 ± 0.1	3.3 ± 0.2	9
Furosemide	1.0 ± 0.2	0.8 ± 0.1 *	4
Tetrodotoxin	0.5 ± 0.2	3.0 ± 0.3	5

* $P < 0.001$ vs. control (no pretreatment).

in many cell types that there is rapid intracellular reduction of vanadate (VO_3^-) to vanadyl (VO^{2+}), which in contrast to vanadate has little if any inhibitory effect on Na^+,K^+-ATPase activity [1,2,10–12]. The lack of a significant inhibitory effect of vanadate on active Na^+ transport in colonic epithelia therefore suggests that vanadate is rapidly reduced to vanadyl also in this tissue.

If indeed there is marked intracellular reduction of vanadate to vanadyl, it is possible that vanadyl rather than vanadate is responsible for colonic Cl^- secretion. We have therefore compared the effects of these two

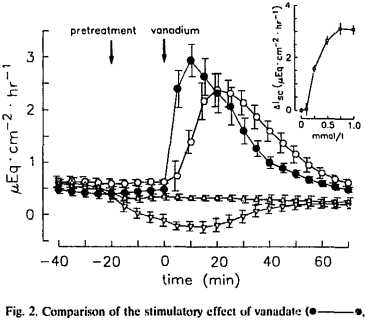

Fig. 2. Comparison of the stimulatory effect of vanadate (●——●, $n = 4$) or vanadyl (○——○, $n = 6$) on I_{sc} across colonic epithelia. Pretreatment with serosal indomethacin (10 μmol/l, ▽——▽, $n = 3$) or quinacrine (0.5 mmol/l, △——△, $n = 3$) abolishes the secretory effect of vanadyl. Both indomethacin and quinacrine were added 20 min before the vanadyl. The inset shows the concentration dependence of the vanadyl effect on I_{sc}, which is half maximal at approximately 0.25 mmol/l. These experiments were performed in the presence of 0.1 mmol/l amiloride in the luminal bathing solution to inhibit electrogenic transepithelial Na^+ transport by inhibition of luminal Na^+ uptake [3].

TABLE II

Effect of serosal addition of vanadate (0.5 mmol/l) or vanadyl (0.5 mmol/l) on the release of PGE_2 into the serosal bathing solution of isolated colonic epithelia in comparison to control (no vanadate or vanadyl)

Pretreatment with indomethacin (10 μmol/l) or quinacrine (0.5 mmol/l), added 20 min before vanadate, abolished the stimulatory effect of vanadate on PGE_2 release. Means ± S.E., n = number of experiments.

	Serosal PGE_2 release (ng 15 min^{-1} cm^{-2})	n
Control	−1.0 ± 1.4	8
Vanadate	291.2 ± 116.3 *	9
Indomethacin + vanadate	−3.8 ± 0.3	5
Quinacrine + vanadate	3.4 ± 0.2	5
Vanadyl	133.5 ± 43.7 *	5

* $P < 0.02$ vs. control.

oxidation states of vanadium on Cl^- secretion (Fig. 2). Vanadyl also stimulated I_{sc}, but the onset of action was slower than after addition of vanadate. This delay of effect most likely is due to the lower uptake rate of the cation vanadyl into the cells compared to that of the anion vanadate [13,14], which appears to be taken up by the anion (phosphate) transport system [12].

Colonic Cl^- secretion is brought about by both a number of local humoral agents (see Ref. 15) and neuronal mechanisms [16]. The enteric nervous system, however, does not seem to be involved in vanadate-induced Cl^- secretion, since the neuronal blocker tetrodotoxin did not inhibit the vanadate effect (Table I). But blocking prostaglandin synthesis with indomethacin, a cyclooxygenase inhibitor [17], or quinacrine, an inhibitor of phospholipase A_2 [18,19], markedly reduced the effects of both vanadyl (Fig. 2) and vanadate (data not shown) on I_{sc}. The notion that prostaglandins mediate the effect of vanadate on Cl^- secretion was substantiated by measurements of prostaglandin E_2 release from the colonic mucosa. Under control conditions no PGE_2 was released during the test period of 15 min. Serosal addition of vanadate or vanadyl caused a large increase of prostaglandin E_2 release into the serosal bathing solution (Table II). The PGE_2 release to the luminal bathing solution was only slightly enhanced by vanadate (data not shown). Pretreatment with indomethacin or quinacrine completely blocked the vanadate-induced release of PGE_2.

The mechanism of epithelial Cl^- secretion involves an increase of intracellular cyclic AMP and/or Ca^{2+} levels, which leads to opening of Cl^- channels in the apical cell membrane (see Ref. 15). There is evidence that vanadate stimulates adenylyl cyclase [20–22]. This prompted Hatch et al. [4] to propose that vanadate elicits intestinal Cl^- secretion by direct activation of adenylyl cyclase. However, our results indicate that the effects of vanadate and vanadyl on Cl^- secretion are mediated by prostaglandins, as both species of vanadium markedly increased the serosal release of prostaglandin E_2, and inhibition of prostaglandin synthesis abolished both stimulated PGE_2 release and Cl^- secretion. Prostaglandins are potent colonic secretagogues [23,24]. It is unclear at present, whether vanadate and vanadyl activate phospholipase A_2 directly via a G protein or indirectly by increasing cell Ca^{2+} [25].

Conclusion

Our experiments provide evidence that vanadate induces Cl^- secretion in rabbit descending colon by stimulating prostaglandin release. Hence, stimulation of prostaglandin release has to be added to the many other actions of vanadate, including inhibition of various phosphohydrolases and phosphotyrosine phosphatases, stimulation of adenylyl cyclase and specific protein kinases, vasoconstriction and regulation of carbohydrate metabolism, cell proliferation and differentiation, and gene expression [1,2,26]. With regard to the possible biological significance of the stimulatory effects of vanadate or vanadyl ions on intestinal secretion, the range of plasma vanadium concentrations in humans has to be considered. Comparing this range, $3 \cdot 10^{-10}$ to $1 \cdot 10^{-5}$ mol/l (see Ref. 1), with the concentrations of vanadate that are necessary for half-maximal stimulation of colonic Cl^- secretion in vitro, approximately 10^{-7} mol/l [4], it is conceivable that high vanadate levels may cause diarrhea. In sheep loose feces are observed when dietary vanadate load exceeds 200 mg/kg of diet [27], and intravenous infusion of vanadium may result in diarrhea in animals (see Ref. 2).

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