Biochimica et Biophysica Acta, 600 (1980) 838—843

BBA 78847

OXALATE TRANSPORT ACROSS THE ISOLATED RAT COLON

A RE-EXAMINATION

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(Received December 17th, 1979)

Key words: Oxalate transport; Permeability; Ca^{2+}

Summary

A net absorption of oxalate and chloride was observed when isolated, short-circuited segments of rat colon were bathed by a calcium-containing buffer. Removal of calcium promoted a two-fold decrease in transmural resistance, while the net chloride flux was reduced and the net oxalate transport abolished. It was concluded that net oxalate absorption was not observed in previous studies (employing calcium-free buffers) because calcium is required to maintain the integrity of the conductive pathways across colonic epithelia.

Introduction

Increased intestinal absorption of dietary oxalate causing hyperoxaluria has been described in a variety of gastrointestinal disorders which have in common malabsorption of fatty acids and bile salts [1]. Current evidence favors the colon as the principal site for this abnormal oxalate absorption [1,2]. The models postulated to explain enteric hyperoxaluria have been based on both changes in oxalate solubility and in colonic membrane permeability [1—4]. However, any model proposed to explain enhanced absorption of oxalate under pathological conditions requires prior understanding of oxalate transport across the normal colon.

Under certain in vitro experimental conditions, Binder has presented evidence that oxalate transport is passive in all portions of the normal intestine [4]. In his studies, oxalate uptake by rabbit ileal mucosal cells was linearly related to extracellular oxalate concentration and was not affected by reduced

temperature, ouabain or dinitrophenol. Furthermore, everted sacs of rat intestine, including the colon, were unable to concentrate oxalate. The results of these studies led to the conclusion that intestinal oxalate flux is a passive phenomenon occurring along electrochemical gradients. It is important to note that these studies were conducted in calcium-free Ringer's solution, the use of which was apparently necessary to prevent formation of insoluble calcium oxalate. However, this aspect of the experimental design may have introduced an artifact affecting the results, since previous studies have shown that removal of calcium causes a significant increase in epithelial membrane permeability [5—7].

Therefore, in order to define more precisely oxalate movement across the colonic epithelium under physiological conditions, we have re-examined the transport of oxalate and chloride in isolated, short-circuited preparations of rat colonic mucosa in both the presence and absence of calcium. The present results indicate that calcium is required to maintain normal permeability of this tissue, and that under short-circuit conditions, a net mucosal-to-serosal transport of oxalate can be observed.

Materials and Methods

Non-fasting, male Sprague-Dawley rats (200—300 g) were anesthesized with pentobarbital. The entire colon was removed, rinsed with the appropriate Ringer's solution, and stripped of its serosal layers as described by Binder and Rawlins [8]. Pieces of colonic mucosa were then mounted in modified Ussing chambers having exposed areas of 1.13 cm^2. Both sides of the tissue were bathed by Ringer's solution, aerated with a 95% O_2/5% CO_2 gas mixture and maintained at 37°C. The composition of the standard Ringer's solution was (concentrations in mmol/l): 141.8 Na^+, 4.5 K^+, 1.2 Ca^{2+}, 1.0 Mg^{2+}, 120 Cl^-, 1.0 SO_4^{2-}, 1.8 HPO_4^{1-}, 0.2 $H_2PO_4^{2-}$, 25.0 HCO_3^-, and 12.0 glucose. Ca^{2+}-free Ringer's solution was prepared by substituting Na^+ for Ca^{2+} in the standard Ringer's solution. Both solutions have pH values between 7.3 and 7.5 under experimental conditions.

Tissues were short-circuited by automatic voltage-clamping devices (WP Instruments DVC100) which compensated for the solution resistance between the voltage-sensing electrodes and the tissue. Tissue conductance (G_T, $m\Omega^{-1}/cm^2$) was calculated from the measured short-circuit current (I_{sc}, $\mu A/cm^2$) and the open-circuit transepithelial potential (V, mV) measured 5—10 s after the clamp current was turned off.

The transepithelial fluxes of chloride and oxalate were measured simultaneously under short-circuit conditions using ^{36}Cl (New England Nuclear) and [^{14}C]oxalate (Amersham). In order to prevent formation of calcium oxalate, carrier-free tracer was used at a final concentration of 1.7 μM. This concentration was low enough to prevent formation of calcium oxalate as calculated from the solubility product for this compound [9]. An equal amount of sodium oxalate was added to the unlabeled side of the tissue.

Unidirectional fluxes were measured for 60—80 min following an initial 20 min equilibration period. At 20 min intervals 0.20 ml samples were removed from the unlabeled side, added directly to a scintillation cocktail, and subse-

TABLE I

ELECTRICAL CHARACTERISTICS OF RAT COLONIC MUCOSA IN THE PRESENCE AND ABSENCE OF CALCIUM

Results are expressed as mean ± S.E. ($\bar{X}$ ± S.E.) based on N tissues or tissue pairs studied. The difference between the means was evaluated by a t-test for unpaired variates.

Condition	V (mV) *	I_{sc} ($\mu A/cm^2$)	G_T ($m\Omega^{-1}/cm^2$)
Normal Ringer's	7.5 ± 1.0 (15)	95.7 ± 10.5 (15)	11.6 ± 1.2 (15)
Ca^{2+}-free Ringer's	1.0 ± 0.1 ** (9)	35.7 ± 7.0 ** (9)	22.4 ± 5.4 ** (8)

* Serosal side positive.

** Significantly different from control, $P < 0.05$.

quently analyzed in a liquid scintillation spectrometer (Tracor, Mark III). Net solute fluxes were computed from the measured differences in unidirectional fluxes of adjacent pieces of tissue from the same animal.

Results

The electrical characteristics of rat colonic mucosa bathed by standard and by Ca^{2+}-free Ringer's solutions are presented in Table I. The values given in this and the following table represent the means for tissues measured at 20 min intervals. As shown in Table I, removal of calcium produced a 1.9-fold increase in membrane conductance, although membrane short-circuit current was significantly reduced.

The unidirectional and net fluxes for Cl^- and oxalate are presented in Table II for tissues in normal and Ca^{2+}-free Ringer's solutions. It may be seen that the removal of calcium resulted in a significant increase in unidirectional fluxes of chloride and oxalate in both directions. It should also be noted that the net absorption of chloride was reduced and that of oxalate abolished when calcium was removed from the Ringer's solution.

Discussion

Rat colon has been previously characterized in vitro as a leaky epithelium ($R_T \approx 80\Omega \cdot cm^2$) capable of absorbing chloride under short-circuit conditions [8,10]. The present observations using a calcium Ringer's solution are in good agreement with these earlier studies. However, it was found that the undirectional fluxes for chloride are lower and the short-circuit current is significantly higher than those reported by Binder and Rawlins [8]. These differences may simply reflect seasonal or group variations between experimental animals, since the calculated conductance values are identical for both groups.

The net absorption of oxalate reported here contrasts with Binder's observations that this solute moves only passively across across intestinal epithelium * [4]. The most significant difference between his study and this report

* We have also consistently observed that there is a net mucosal-to-serosal transport of oxalate across the isolated, short-circuited rabbit colon in the presence of calcium. A detailed consideration of these observations will be presented elsewhere.

TABLE II

UNIDIRECTIONAL AND NET FLUXES OF CHLORIDE AND OXALATE ACROSS RAT COLONIC MUCOSA IN THE PRESENCE AND ABSENCE OF CALCIUM

Results are expressed as mean ± S.E. ($\bar{X}$ ± S.E.) based on N tissues or tissues pairs studied. The difference between the means was evaluated by a t-test.

Condition	J^{Cl}_{sm} (μequiv./cm^2 per h)	J^{CL}_{ms} (μequiv./cm^2 per h)	J^{Cl}_{net} (pmol/cm^2 per h)	J^{ox}_{sm} (pmol/cm^2 per h)	J^{ox}_{ms} (pmol/cm^2 per h)	J^{ox}_{net} * (pmol/cm^2 per h)
Normal Ringer's	8.7 ± 0.3 (6)	16.7 ± 1.2 (8)	7.3 ± 1.3 (6)	33.4 ± 3.9 (6)	54.8 ± 7.9 (8)	21.2 ± 7.5 (6)
Ca^{2+}-free Ringer's	15.9 ± 0.7 (6)	19.6 ± 0.8 (6)	3.5 ± 0.4 (6)	80.3 ± 9.3 (6)	75.0 ± 9.3 (6)	−2.9 ± 6.6 (6)
P	<0.01	<0.10	<0.05	<0.01	<0.10	<0.05

* A negative net flux indicates secretion.

is that calcium was not present in the Ringer's solutions which Binder used. Exclusion of calcium was presumably done to avoid the formation of insoluble calcium oxalate. As shown here, the removal of calcium promotes an overall increase in tissue electrical conductance which is accompanied by significant increases in the unidirectional fluxes of both chloride and oxalate. Furthermore, calcium removal abolished net flux of oxalate and reduced absorption of chloride by half.

It is conceivable that calcium removal could affect both active transcellular solute fluxes as well as ion flows through paracellular or other shunt pathways. Some transcellular electrolyte flux must be altered in Ca^{2+}-free Ringer's solution, since I_{sc} was markedly reduced under these conditions. However, the 2-fold increase in tissue conductance, in spite of the fall in short-circuit current, suggests that some shunt pathway is the more sensitive component of tissue conductance. Whether this increase in shunt conductance is transcellular or paracellular cannot be definitively resolved; however, the persistence of a net chloride absorption, despite a nearly 2-fold increase in J_{sm}^{Cl} suggests that the conductance increase is not simply due to cell membrane alteration, but that junctional permeability must also be significantly calcium sensitive. This conclusion is in accordance with the general observations that the conductance properties of 'leaky' epithelia, like the rat colon, are determined largely by paracellular pathways [11,12], and that Ca^{2+} is involved in maintaining the integrity of junctional regions [6].

The magnitude of both net and unidirectional fluxes of oxalate reported here are quite low due to the fact that no significant amount of carrier oxalate can be employed to measure oxalate transport in the presence of calcium. Although there are potential complications in interpreting tracer fluxes in terms of permeability coefficients under the present experimental conditions [13], at least the relative changes in oxalate absorption may be ascribed to the biological effects of calcium removal. If it is assumed that, in normal salines, the flux of oxalate through the serosal-to-mucosal pathway is independent of the flows of other solute species, then a permeability coefficient for oxalate may be calculated from the relation $P_{sm}^{ox} = J_{sm}^{ox}/C^{ox}$ [14]. In the present experiment, $P_{sm}^{ox} = 0.6 \cdot 10^{-5}$ cm/s, while $P_{sm}^{Cl} = 2.0 \cdot 10^{-5}$ cm/s. The finding that P_{sm}^{ox} is some 4-times smaller than that for simultaneously measured P_{sm}^{Cl} may reflect electrostatic as well as steric differences between these anions. The shunt pathway in another 'leaky' epithelium, the proximal tubule of *Necturus* kidney, also displays a selectivity of chloride over organic anions [15]. In this tissue the ratios P^{anion}/P^{Cl} for both propionate and acetate were about 0.3, which is the same as observed here with respect to oxalate and chloride.

These studies have demonstrated the possibility of an active component of oxalate transport across the normal rat colon. The in vivo consequences of active oxalate absorption are likely to be more significant under conditions where only traces of dietary oxalate are in solution and available for accumulation. However, under pathological conditions, such as those accompanying fatty acid or bile salt malabsorption [1], passive oxalate transport along electrochemical gradients [3] may become more significant than the net transfer process described above.

Acknowledgement

This work was supported in part by NIH Grant HL 07249.

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