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In Vitro Studies of Sodium Transport in Human Infant Colon: The Influence of Acetate

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ABSTRACT. In infants, adequate colonic function is vital in preventing electrolyte and water depletion. In certain species, short-chain fatty acids have been shown to increase colonic Na absorption. Using an *in vitro* voltage-clamp technique, we have studied the characteristics of electrolyte transport in isolated preparations of human left-sided colonic mucosa and investigated the effect of acetate on epithelial Na movement. In the basal state, there was net Na absorption that was entirely electrogenic. The addition of mucosal acetate resulted in a significant increase in net Na absorption that was markedly inhibited by amiloride, suggesting that, in the young child, the presence of short-chain fatty acids promotes colonic salvage of Na and that such salvage of Na may be via an amiloride-sensitive Na channel and involve stimulation of sodium-hydrogen exchange. (*Pediatr Res* 34: 666–669, 1993)

Abbreviations

SCFA, short-chain fatty acid
Ac, acetate
G, conductance
PD, potential difference
Isc, short-circuit current
Cl, chloride
JNanet, net flux of sodium ions
JNams, mucosa to serosa flux of sodium ions

Although it is clear that the majority of water and electrolyte absorption occurs in the small intestine, it is often the adequacy of colonic function that determines whether or not there is diarrhea and consequent net loss of water and electrolytes from the body (1). *In vivo* studies in both the experimental animal (2–4) and the human colon (5–8) have established that Na and Cl are absorbed and that K and HCO_3 are secreted. *In vitro* studies, in which both the chemical and spontaneous electrical gradients are controlled, have provided important additional information regarding the mechanisms responsible for electrolyte movement across colonic epithelia in the adult human (9–16).

SCFA, which are produced by colonic bacterial fermentation of dietary carbohydrates, are the principal anions found in the colon of several mammalian species and may be important in the salvage of both calories and electrolytes. Indeed, they influence colonic electrolyte transport (17–24), and *in vivo* studies have provided evidence that SCFA are rapidly absorbed from the colon of man (25) and that they can promote Na and water absorption from the colon (26, 27).

The only data on possible mechanisms of colonic salt and water absorption in human infants are derived from *in vivo* studies where electrical gradients influence ion movements and chemical gradients are uncontrolled (8). The purpose of our present study was to carry out a more detailed analysis of Na transport in isolated human infant colon using a voltage clamp technique in an Ussing chamber, and also to investigate the effect on colonic Na absorption of adding mucosal Ac.

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MATERIALS AND METHODS

Human colon preparation. Colonic tissue was obtained from children undergoing colonic operation for Hirschsprung's disease or anal atresia at the Hospital for Sick Children. Tissue whose resistance was lower than 90 ohms/cm^2 was discarded. Ten tissue pairs were studied from the left colons of eight different children, aged 10–20 mo (four with Hirschsprung's disease and four with anal atresia). The segments of descending colon, of lengths varying from 4 to 20 cm, were removed by the surgeon immediately after compromising the blood supply and then opened along the mesenteric border and immersed in iced, preoxygenated Krebs-Hensleit solution. Muscle coats were removed from the intestine, as previously described, before mounting in the chambers.

The specimens of mucosa, which were all macroscopically normal (confirmed by subsequent histologic examination), were then mounted between two Perspex half chambers within 10–15 min of removal from the body, as previously described (28). An area of 0.64 cm^2 of each surface of the mucosa was exposed to 15 mL of Krebs-Hensleit bathing solution containing Na 143, K 5.9, Ca 1.9, Mg 1.1, Cl 129, HPO_4 1.2, HCO_3 25, and glucose 10 mmol/L (pH 7.0). The solutions were maintained at 37°C by heated water jackets and were oxygenated and circulated via a bubble-lift mechanism containing a 95% O_2-5% CO_2 mixture.

Electrical measurements. The spontaneous transmucosal electrical PD was measured through 2% agar-salt bridges containing Krebs-Hensleit solution connected to a high-impedance digital voltmeter (Analogic AN 2570) via matched calomel electrodes (asymmetric potential ±0.2 mV) immersed in 3 M KCl. The combined electrode and bridge junction potentials did not exceed 0.5 mV. The mucosa was short-circuited by a current (Isc) passed from an external dry battery via Ag/Ag Cl electrodes and agar salt bridges placed at opposite ends of the half chambers. The current was adjusted manually every 2–3 min and a correction was made for the drop in potential caused by the resistance of the fluid gap between the PD electrode tips, as described by Field *et al.* (29).

The electrical resistance of the mucosa, and thus G, was determined by passing a pulse of direct current of 100 μA and correcting the change in PD for fluid resistance. An initial fall in PD of 2–3 mV occurred after mounting the tissue in the chamber, but after 20 min PD, Isc, and G had reached values that remained stable for 180 min before gradually declining.

Radioisotope fluxes. Fluxes of radiolabeled Na were measured on paired tissues taken from adjacent segments of colon, and either two or four specimens were set up for each experiment.

Tissues whose electrical resistance differed by less than 25% were paired to determine unidirectional fluxes. Twenty min after mounting the tissue to the mucosal reservoir of one chamber and to the serosal reservoir of the other chamber for each pair of tissues, 2.2 μCi of each isotope were added. The tissues were then short-circuited for 20 min, after which a 2-mL sample was removed from each unlabeled bathing solution and a 100-μL sample from each labeled solution. Samples removed from the unlabeled solution were replaced by an equal volume of the appropriate unlabeled bathing solution. Duplicate samples were taken 20 min later to determine baseline flux rates.

After this, in four pairs of tissue, amiloride 10^{-4} M was added to the mucosal surface and, after a further 20-min equilibration period, flux experiments were again performed. In the other six pairs of tissue, after the basal flux experiments had been completed, the original bathing solution on the mucosal side of the tissues was replaced by a solution containing 60 mmol of Ac and 69 mmol of Cl, the composition of the fluid being otherwise identical to the original solution. After a 20-min equilibration period, further Na flux experiments were performed replacing the samples taken with solutions of appropriate electrolyte content. Amiloride 10^{-4} M was then added to the mucosal surface and, after a further 20-min equilibration period, final Na flux experiments were undertaken.

The samples were counted and calculations were performed as described by Booth *et al.* (28). All values are expressed as median (range) and, in experiments involving both Ac and amiloride, statistical comparisons were made using a modification of the multiple comparisons procedure of Friedman (30).

It is clear that in the experiments involving Ac both Ac and Cl are asymmetric across the tissue and gradients in opposite directions exist for each of them. A Cl diffusion potential will obviously exist. However, it is unknown whether there is a significant diffusion of SCFA across human colon, and there are conflicting data from animal experiments (31, 32). If there is a significant diffusion of SCFA, then it might be expected that this would cancel out any diffusion potential generated by the passive diffusion of Cl ions in the opposite direction. We have thus expressed the results without taking into account any possible diffusion potential.

RESULTS

Table 1 shows the results of individual basal flux experiments in 10 pairs of left-sided colon, and Table 2 shows the flux results in four pairs of colon before and after the addition of mucosal amiloride.

It is clear that, under basal conditions, there was net Na absorption and JNanet closely approximated Isc. The addition of amiloride markedly reduced JNanet via an effect on JNams and almost abolished Isc, with a concomitant fall in G. These results suggest that Na absorption in infant left colon under basal conditions occurs largely via an electrogenic, amiloride-sensitive pathway that can account almost entirely for the measured Isc.

Table 3 shows the results obtained from Na flux experiments expressed as median (range) in six pairs of tissue, first under basal conditions, then after the addition of mucosal Ac 60 $\times$ 10^{-3} M and again after the addition of mucosal amiloride 10^{-4} M. After the addition of Ac there was a highly significant increase in Na absorption, due entirely to an increase in JNams. There was no change in Isc or PD. The addition of amiloride reduced JNams (and thus JNanet) to levels seen in the basal state before the addition of acetate and resulted in a reduction in Isc and PD.

DISCUSSION

The large intestine plays a particular role in the salvage of salt, water, and partially digested nutrients. In infancy, colonic transport mechanisms are particularly important in Na conservation (8), but no *in vitro* systematic studies of colonic Na absorption have been previously undertaken in childhood. A recent study has reported that the mechanism of Na absorption may vary in different parts of the colon (14), but unfortunately we were only able to obtain satisfactory tissue from the left side of the colon.

In vivo perfusion studies in adult humans have suggested that SCFA, derived from bacterial fermentation of carbohydrate, may promote Na and water absorption, but this has not been previously demonstrated *in vitro* nor has the mechanism been defined. However, *in vitro* studies of adult human colon (9–16) have shown that there is active Na absorption that may entirely account for the Isc (12, 14), although some studies observed that JNanet may be greater than Isc (9–11, 13, 15). Our data show that JNanet approximates Isc and is largely abolished by amiloride, suggesting that, in infant descending colon, active Na absorption occurs mostly via an electrogenic, amiloride-sensitive process that is responsible for the generation of transmural PD and Isc. We have recently shown that rectal Na absorption in infants is closely related to circulating aldosterone levels (8). The finding of an electrogenic, amiloride-sensitive pathway in the present study is entirely in keeping with perhaps the major effect of aldosterone on colonic Na transport as an Na regulatory hormone (33).

Although four of the patients studied suffered from Hirschsprung's disease, it has been previously shown that aganglionic colon is an efficient absorptive epithelium (34) and our *in vitro* studies now confirm this. In our experiments, basal Isc and G were similar to those reported in adult human colon, although PD was lower (9–16).

Perfusion studies in adult colon have shown that Na absorption increased in the presence of SCFA in the lumen via a process as yet unexplained (27, 35). It has been postulated that this may occur via the recycling of H ions and the presence of an Na/H antiporter. Our basal flux data show little evidence of such an antiporter because JNanet only slightly exceeds Isc. However, in our study it is possible that the stimulation of Na absorption

Table 1. *Basal sodium flux, transepithelial PD, tissue G, and Isc in 10 pairs of human infant left colon**

Pair	PD (mV)	G (mS/cm^2)	Isc (μmol/h/cm^2)	Jms (μmol/h/cm^2)	Jsm (μmol/h/cm^2)	Jnet (μmol/h/cm^2)
1	9.7	6.1	3.5	7.67	0.9	+6.77
2	7.9	9.4	4.61	7.58	1.43	+6.15
3	4.7	9.3	2.62	10.43	5.62	+4.81
4	7.7	8.3	3.56	7.84	3.31	+4.53
5	7.4	8.0	3.56	3.48	2.85	+0.63
6	7.3	10.9	4.55	8.66	7.61	+1.05
7	7.8	7.8	4.2	6.4	2.5	+3.9
8	8.1	7.3	3.9	8.32	5.91	+2.41
9	5.8	9.1	2.6	6.45	3.56	+2.89
10	7.1	9.0	2.9	6.47	2.87	+3.6
Median values	7.55	8.6	3.56	7.63	3.09	+3.75

* Jms, mucosa to serosa flux; Jsm, serosa to mucosa flux; Jnet, net flux; +, net absorption.

Table 2. *Na flux, PD, G, and Isc in four pairs of human infant left colon in basal (B) state and after addition of mucosal amiloride 10^{-4} M (Am)**

Pair	PD (mV)	G (mS/cm²)	Isc (μmol/h/cm²)	Jms (μmol/h/cm²)	Jsm (μmol/h/cm²)	Jnet (μmol/h/cm²)†
1						
B	7.8	7.8	4.2	6.4	2.5	+3.9
Am	2.3	6.6	0.9	4.2	2.8	+1.4
2						
B	8.1	7.3	3.9	8.32	5.91	+2.41
Am	3.5	5.8	0.4	5.6	5.69	−0.09
3						
B	5.8	9.1	2.6	6.45	3.56	+2.89
Am	2.4	7.2	0.1	3.4	3.68	−0.28
4						
B	7.1	9.0	2.9	6.47	2.87	+3.6
Am	2.6	6.8	0.2	3.7	3.43	+0.27
Median values						
B	7.45	8.3	3.4	6.46	3.22	+3.25
Am	2.5	6.6	0.3	3.95	3.56	+0.09

* Abbreviations are the same as in Table 1.
† + = net absorption, − = net secretion.

Table 3. *Na flux, PD, G, and Isc in six pairs of human infant left colon in basal state (B), after addition of mucosal Ac 60×10^{-3} M, and then after addition of mucosal amiloride 10^{-4} M (Am)**

	PD (mV)	G (mS/cm²)	Isc (μmol/h/cm²)	Jms (μmol/h/cm²)	Jsm (μmol/h/cm²)	Jnet (μmol/h/cm²)
B	7.55 (4.7–9.7)	8.7 (6.1–10.9)	3.56 (2.62–4.61)	7.76 (3.48–10.43)	3.08 (0.9–7.61)	+4.67 (+0.63–+6.77)
Ac	8.6 (6.5–10.7)	7.0 (5–9.5)	3.85 (2.92–4.84)	13.29 (9.58–20.6)	4.71 (1.47–9.65)	+9.17 (−0.07–+16.1)
Am	3.6 (0–7.8)	6.5 (5–8.3)	1.34 (0–3.56)	8.31 (3.73–10.85)	5.36 (1.48–9.1)	+4.1 (−5.37–+5.79)
p B *vs* Ac	NS	NS	NS	<0.05	NS	<0.05
p Ac *vs* Am	<0.05	NS	<0.05	<0.05	NS	<0.05

* Results are shown as median and range and *p* denotes the significance value between B and Ac and then Ac and Am results using the multiple comparisons procedure of Friedman. Abbreviations are the same as in Table 1.

seen in the presence of acetate might be due to the unmasking of Na/H exchange, which might then operate in concert with the electrogenic Na absorption existing in the basal state. The addition of amiloride might then inhibit the electrogenic pathway for Na absorption with a lesser effect on Na/H exchange and thus explain why amiloride only partially reduced, rather than abolished, JNanet.

A second hypothesis has been advanced to explain the effect of SCFA on Na absorption that suggests that the stimulation of Na transport relates to the ionic diffusion of Ac anions. Indeed, at the pH of both colonic luminal contents (pH 6–7) and the microclimate of the epithelial cell (pH 5.8–6.0), SCFA (dissociation constant 4.8) will be dissociated. Our data are also consistent with this theory, because one explanation for the increased Na absorption is that an equivalent amount of Ac is absorbed with the Na. If any Cl diffusion potential is balanced by an equal but opposite Ac diffusion potential, then the lack of change in PD and Isc is explicable using the above hypotheses. In the absence of definite data regarding the passive permeability of Ac across human childhood colonic epithelium and considering that data from animal studies are conflicting (31, 32), it is difficult to accurately interpret the PD and Isc recorded after the addition of Ac. It is, however, likely that stimulation of Na/H exchange is involved, as has been shown in other animal experiments.

Thus, although the precise mechanism involved is unknown, there is no doubt that Na absorption by infant left colon occurs in the basal state largely by an amiloride-sensitive electrogenic Na-absorptive mechanism. The presence of mucosal acetate increases Na absorption probably by the stimulation of Na/H exchange, which is not evident under basal study conditions. It is therefore clear that the bacterial metabolism of carbohydrate in the colon may be an important factor in the overall salvage of salt and water in the young infant.

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