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Research Article

Regional variations in the ion transport properties of the colon may have significant physiological and pathophysiological implications. However, only limited studies have been performed in cecum, which comprises 50% of the macrosurface of the rabbit colon. In vitro under short-circuit conditions, cecum actively absorbed Na and Cl (JnetNa = 5.6 +/- 0.3, JnetCl = 1.5 +/- 0.3 mu eq.cm-2.h-1) with a short-circuit current (Isc) of 6.29 +/- 0.2 mu eq.cm-2.h-1.Cl substitution with sulfate decreased both JnetNa and Isc by 1.3 mu eq/cm2.h-1.HCO3 removal decreased both JnetNa and Isc 3.3 mu eq.cm-2.h-1. This effect was due primarily to removal of serosal HCO3. There was both a linear correlation between JNanet and Isc (r = 0.845) and a concentration-dependent stimulation of Isc by increasing [Na] in the bathing media. However, 10(-4) M amiloride did not significantly alter either Isc or JnetNa. In contrast, 10(-4) M phenamil, an amiloride analogue highly specific for the Na channel, significantly blocked both Isc and JnetNa. The sulfhydryl reagent PCMBS increased Isc; this response was reversed by phenamil. Electrogenic Cl secretion was stimulated by 1 mM theophylline, 10(-4) M 8BrcAMP and 10(-4) M 8BrcGMP. None of the secretagogues inhibited JnetNa. Epinephrine (5.5 microM) increased JnetNa from 5.9 +/- 1.3 to 7.8 +/- 1.1 (P = 0.02) and JnetCl from 0.1 +/- 1.2 to 2.0 +/- 0.8 (P […]

Electrogenic Sodium Absorption in Rabbit Cecum In Vitro

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Abstract

Regional variations in the ion transport properties of the colon may have significant physiological and pathophysiological implications. However, only limited studies have been performed in cecum, which comprises 50% of the macrosurface of the rabbit colon. In vitro under short-circuit conditions, cecum actively absorbed Na and Cl (J^{Na}_{net} = 5.6±0.3, J^{Cl}_{net} = 1.5±0.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) with a short-circuit current (I_{sc}) of 6.29±0.2 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. Cl substitution with sulfate decreased both J^{Na}_{net} and I_{sc} by 1.3 $\mu eq/cm^2 \cdot h^{-1}$. HCO_3 removal decreased both J^{Na}_{net} and I_{sc} 3.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. This effect was due primarily to removal of serosal HCO_3. There was both a linear correlation between J^{Na}_{net} and I_{sc} (r = 0.845) and a concentration-dependent stimulation of I_{sc} by increasing [Na] in the bathing media. However, 10^{-4} M amiloride did not significantly alter either I_{sc} or J^{Na}_{net}. In contrast, 10^{-4} M phenamil, an amiloride analogue highly specific for the Na channel, significantly blocked both I_{sc} and J^{Na}_{net}. The sulfhydryl reagent PCMBS increased I_{sc}; this response was reversed by phenamil. Electrogenic Cl secretion was stimulated by 1 mM theophylline, 10^{-4} M 8BrcAMP and 10^{-4} M 8BrcGMP. None of the secretagogues inhibited J^{Na}_{net}. Epinephrine (5.5 μM) increased J^{Na}_{net} from 5.9±1.3 to 7.8±1.1 (P = 0.02) and J^{Cl}_{net} from 0.1±1.2 to 2.0±0.8 (P NS) $\mu eq \cdot cm^{-2} \cdot h^{-1}$. Studies of pH stat demonstrated an epinephrine-stimulated increase in $J^{HCO_3}_{m-s}$ without a change in $J^{HCO_3}_{s-m}$. Thus, cecum exhibits a distinct type of electrogenic Na electrogenic Na absorption which is partially dependent on the presence of Cl and HCO_3, not blocked by amiloride but by phenamil. Because of its large surface area and its novel mechanism of electrogenic Na transport, the cecum exerts an important regulatory role in colonic fluid and electrolyte balance.

Introduction

Segmental heterogeneity of intestinal epithelial electrolyte transport may underlie important functional differences and provide insight into the integrative function of the gut. Recent studies from our laboratory and others have established that there are important differences between the proximal and distal colon (1–4). Additionally, there now appear to be differences within the rabbit proximal colon between the segment immediately distal to the cecum (the triple haustrated segment) and the region 10 cm beyond the cecum (the single haustrated segment) (4, 5).

The cecum's transport function, however, has remained largely uninvestigated. Limited studies have suggested that the cecum absorbs Na efficiently (4, 6). In the present study, we characterize in detail the ion transport characteristics of rabbit cecum in vitro.

Cecal transport is dominated by electrogenic Na transport that is not readily inhibited by amiloride. However, the amiloride analogue phenamil has a significant inhibitory effect. Na absorption is not obviously coupled to Cl or HCO_3, but is somewhat dependent on the presence of both anions. Electrogenic Cl secretion can be stimulated by both cAMP and cGMP-mediated secretagogues, but there is no inhibition of electroneutral NaCl absorption. Epinephrine elicits both electroneutral Na absorption and HCO_3 absorption. Given the Na absorptive capacity and relatively large surface area of the cecum, this colonic segment plays an important role in intestinal fluid and electrolyte balance.

Methods

Experimental protocol. New Zealand White male rabbits (2–3 kg) fed with standard rabbit laboratory diet and water *ad libitum* were killed during the soft feces period with an i.v. injection (3.0 ml/kg rabbit) of enthanasia solution T6 in an ear marginal vein. (*N*-[2-(M-methoxyphenyl)-2 ethyl-butyl-(1)]-gamma hydroxy butyramide, 200 mg/ml; 4,4′ methylene-*bis*(cycholexyl)trimethyl-ammonium iodide), 50 mg/ml; tetracaine HCl, 5 mg/ml, 0.6 ml dimethylformamide). A 10-cm segment of cecum between the appendix and the ileo-cecal valve was rapidly excised, opened along its mesenteric border, and rinsed several times in an ice-cold bathing solution gassed with 95% O_2, 5% CO_2, until its surface was clear of feces. The segment was then placed serosa side up on a plexiglass plate; transverse incisions were made on every other colonic haustra with a razor blade. Two-haustrated small segments were returned rapidly to the bubbled solution. The serosa and outer layer were dissected with a fine forcep from each one of the two-haustrated segments.

Ringer standard solutions used in these experiments contained (in mmol/liter): NaCl, 114; KCl, 5; Na H_2PO_4, 0.30; $NaHPO_4$, 1.65; $CaCl_2$, 1.25; $MgCl_2$, 1.1; $NaHCO_3$, 25; glucose, 10. Either choline or lithium replaced Na in Na-free solutions; sulfate and mannitol replaced chloride. HCO_3-free solutions had 5 mM Hepes as a substitute buffer.

Transepithelial electrical potential difference (PD),[1] total conductance (G_t), and short-circuit current (I_{sc}) were measured as described previously (1). Pieces of intestinal mucosa were mounted in Ussing chambers (1.12 cm^2 of exposed surface area) and bathed with 10 ml of standard Ringer's solution on each side. Solutions were circulated by gas lift and maintained at 37°C in water-jacketed reservoirs. In HCO_3-buffered solutions, 95% O_2/5% CO_2 was employed; in HCO_3-free experiments, 100% O_2 was used.

Ion fluxes were measured over two successive periods. Tissues from the same rabbit were mounted for 45 min before ^{22}Na and ^{36}Cl were added together to either the mucosal or serosal reservoirs, and the

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1. *Abbreviations used in this paper:* 8BrcAMP, 8-bromo-cAMP; 8BrcGMP, 8-bromo-cGMP; G_t, total conductance; I_{sc}, short-circuit current; J_{m-s}, J_{s-m}, mucosal-to-serosal and serosal-to-mucosal fluxes; PCMBS, *P*-hydroxymercuribenzoate sodium; PD, potential difference.

tissues were short-circuited by voltage clamping. Tissues were paired by matching resistances. If at any time during flux measurements the resistances of paired tissues differed by > 25%, the experiment was rejected. The initial flux (period 1) lasted 30 min; after a 20-min equilibration period, a second flux measurement was made over a 40-min period (period II). Test substances were added 10–15 min before a flux period, depending on the protocol of the individual experiment. Electrical responses were assessed during the 15 min after addition of a specific agent when fluxes were not performed.

Unidirectional mucosal-to-serosal and serosal-to-mucosal fluxes ($J_{m\text{-}s}$ and $J_{s\text{-}m}$) and the net fluxes (J_{net}) of Na, K, and Cl were calculated from aliquots taken at the beginning and end of each flux period. To calculate the unidirectional ion fluxes, we divided the steady-state rates of radioisotope transfer by the specific activity of the initially labeled side and by the surface area of exposed tissue. The net flux is calculated as the difference between oppositely directed unidirectional fluxes of tissue pairs ($J_{net} = J_{m\text{-}s} - J_{s\text{-}m}$). From these measurements, the residual ion flux ($J^R = I_{sc} - J^{Na}_{net} + J^{Cl}_{net}$), which represents that part of the I_{sc} not attributable to the movement of Na or Cl, was calculated.

pH stat experiments. Two segments of stripped cecal mucosa were mounted in Ussing chambers; the transepithelial electrical potential difference referenced to the serosal bathing solution, I_{sc}, and conductance (G_t) were determined. Rates of luminal acidification/alkalinization ($J_{s\text{-}m}$) were determined by using the HCO_3-Ringer's as the serosal solution and a Ringer's solution in which all bicarbonate and phosphates were replaced with 28 mM Na-gluconate and 1 mM Hepes in the mucosal reservoir. The serosal solution was gassed with 95% O_2/5% CO_2 and, at 37°C, had a pH of 7.4. The mucosal solution was gassed with 100% O_2 which passed through an Ascarite II CO_2 trap (Thomas Scientific, Swedesboro, NJ) and pH was maintained constant at pH 7.4 with a pH stat apparatus (Radiometer, Copenhagen, Denmark) under short-circuit conditions. The titrants used were 0.01 N HCl or NaOH in phosphate-bicarbonate–free Ringer's previously described.

Rates of serosal ($J_{m\text{-}s}$) acidification/alkalinization were determined by mounting tissues with the serosal surface bathed in the phosphate-bicarbonate–free Ringer's and the mucosal surface bathed with standard HCO_3-Ringer's. After the tissues had stabilized electrically, rates of acidification/alkalinization were determined over a 30–60-min period.

The effects of epinephrine on acidification/alkalinization were studied on both mucosal and serosal surfaces of the rabbit cecum as follows: (*a*) tissues were mounted in vitro and allowed to reach an electrical steady state; (*b*) a control (period I) flux of 30–40 min was measured; and (*c*) epinephrine adjusted to pH 7.4 (ESI Pharmaceuticals, Elkins-Sinn, Inc., Cherry Hill, NJ) was added to produce a final concentration of 5.5×10^{-5} M (period II). The period II flux was measured for 30–40 min. This procedure was followed for both m-s and s-m flux measurements.

Although we cannot definitely determine the ionic species responsible for changes in pH, we have arbitrarily decided to express such changes as J^{HCO_3} (in $\mu eq \cdot cm^{-2} \cdot h^{-1}$). J^{HCO_3} was calculated from the amount of titrant added over time. We observed a slow drift in pH of the pH stat solution without tissue (-0.62 ± 0.31 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, $n = 16$). Therefore, the baseline drift of the pH stat solution was determined before and after the experiment. The drift was substracted from the measured rate of acidification/alkalinization to determine calculated rate of HCO_3 movement of the tissue.

Statistics. Student's paired *t* test was used for pair-matched controls in the same animal. Otherwise, Student's unpaired *t* test was employed, except for Table I in which analysis of variance and the Tukey multiple comparison test were employed.

Materials. Epinephrine was obtained from Elkins-Sinn, Inc. (Cherry Hill, NJ). *P*-hydroxymercuribenzoate sodium salt, theophylline, 8BrcAMP, and 8BrcGMP were obtained from Sigma Chemical Co. (St. Louis, MO). Amiloride, phenamil, 5-(*N*-prophyl-*N*-butyl)-2',4' dichlorobenzamil, 5-[*N*-(4-chlorobenzyl)-2',4'-dimethyl-benzamil, and 2'4'-dimethylbenzamil were synthesized by previously established methods (7, 8). ^{22}Na and ^{42}K were obtained from New England Nuclear (Boston, MA); ^{36}Cl from ICN Pharmaceuticals (Cleveland, OH).

Results

Basal ion transport. In vitro rabbit cecum, bathed in normal Ringer's, exhibited a reproducible and characteristic set of transport parameters. There was a significant, serosa-positive I_{sc}, 6.29 ± 0.20 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. This I_{sc} is higher than the basal I_{sc} found in small intestine (9), proximal colon (1), or distal colon (10) implying significant electrogenic ion transport. The conductance of 7.0 ± 0.3 $mS \cdot cm^{-2}$ is somewhat higher than that of distal colon but lower than that found in proximal colon (1, 10), indicating that the cecum is a moderately "tight" epithelium. Ion fluxes demonstrate that the cecum absorbs Na actively at a higher rate than other intestinal segments (Table I). Cl is absorbed at a much lower rate (1.5 ± 0.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) and there is a significant residual ion flux. J^{Na}_{net} equals 89% of basal I_{sc}. This pattern of ion fluxes is similar to distal colon, in which electrogenic Na absorption accounts for I_{sc} and anion transport is mediated primarily by electroneutral Cl-HCO_3 exchange (10).

To determine whether the epithelium in vitro was stable over time, successive flux periods were performed (Table II). There was a significant increase in conductance of 2.7 ± 0.9 $mS \cdot cm^{-2}$ ($P = 0.027$). This was reflected in a trend of increased unidirectional fluxes of both Na and Cl. However,

Table I. Basal Ion Transport Parameters in Rabbit Cecum

Group	J^{Na}			J^{Cl}			I_{sc}	J^R	G_t
	m-s	s-m	net	m-s	s-m	net			
	$\mu eq \cdot cm^{-2} \cdot h^{-1}$								$mS \cdot cm^{-2}$
A. Normal Ringer's (41)	9.2±0.4	3.6±0.2	5.6±0.3	8.2±0.4	6.7±0.3	1.5±0.3	6.29±0.2	2.2±0.4	7.0±0.3
B. Cl-free (9)	6.6±0.8*	2.2±02*	4.4±0.7	—	—	—	4.80±0.5*	0.5±0.5	3.8±0.5*
C. HCO_3-free (9)	4.5±0.4*	2.4±0.4*	2.1±0.3*‡	7.1±0.5	7.6±0.5	−0.5±0.6*	3.11±0.3*‡	0.5±0.6	5.8±0.6*‡
D. NA-free choline (8)	—	—	—	8.1±0.6	7.6±0.5	0.5±0.6	1.11±0.29*‡§	1.5±0.7	9.7±0.7
E. Na-free lithium (4)	—	—	—	ND	ND	ND	0.26±0.02	ND	15.7±3.6*‡§

Composition of solutions is described in Methods. Number of subjects given in parentheses. * $P < 0.05$, compared with group A. ‡ $P < 0.05$ compared with group B. § $P < 0.05$ compared with group C, using Tukey multiple comparison procedure. ND, not done.

Table II. Stability of Transport Parameters Over Time

Group	J^{Na}			J^{Cl}			I_{sc}	J^R	G_t
	m-s	s-m	net	m-s	s-m	net			
	$\mu eq \cdot cm^{-2} \cdot h^{-1}$								$mS \cdot cm^{-2}$
I Control	8.1±0.4	3.2±0.4	4.9±0.4	6.3±0.4	5.1±0.4	1.2±0.5	5.94±0.49	2.2±0.5	6.8±0.5
II Control	8.9±0.7	3.7±0.4	5.3±0.4	6.9±0.5	6.2±0.6	0.7±0.5	6.36±0.49	1.8±0.4	9.3±1.5*

Results are given for eight animals. The protocol for these studies involved an initial basal flux measurement over 30 min (I Control), a 20-min stabilization and a second flux period of 40 min (II Control). * $P < 0.05$ vs. control.

there was neither a significant change in fluxes nor in I_{sc} between the two periods. Thus the transport parameters are stable (save for G_t) for the 150–180 minutes required for in vitro flux studies.

Ion substitutions. To further elucidate the mechanisms of cecal ion transport, flux studies were performed in Na-free Ringer's, Cl-free sulfate Ringer's, and bicarbonate-free, Hepes-buffered Ringer's solution.

We employed two Na-free solutions with either choline or lithium substitution. In the lithium-substituted Ringer's, there was a minimal I_{sc}, 0.27±0.02 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. In the choline-Ringer's, the I_{sc} was decreased from that in normal Ringer's (1.11±0.29 $\mu eq \cdot cm^{-2} \cdot h^{-1}$), but somewhat higher than in the lithium-containing solutions. The explanation for this difference is not readily apparent. In the choline-containing Ringer's, there was no significant Cl net movement.

Under Cl-free conditions, I_{sc} was 4.80±0.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, significantly less than that in normal Ringer's. J^{Na}_{net} was 4.4±0.7 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, also reduced when compared to normal Ringer's. The lower G_t found in the Cl-free solution (3.8±0.5 $mS \cdot cm^{-2}$) suggests that there is a major Cl conductance in the cecum.

In bicarbonate-free Ringer's, I_{sc} was 3.11±0.31 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, significantly less than that in normal Ringer's. J^{Na}_{net} under these conditions was 2.1±0.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, while Cl transport was essentially nil (−0.5±0.6 $\mu eq \cdot cm^{-2} \cdot h^{-1}$). The conductance of 5.8±0.6 $mS \cdot cm^{-2}$ was similar to that in normal Ringer's.

Anions clearly have an important role in regulating Na transport in rabbit cecum. In Cl-free Ringer's, J^{Na}_{net} is reduced by 1.2 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ while in HCO_3-free Ringer's it is decreased by 3.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. This may represent either anion-coupled or anion-dependent Na absorption. In HCO_3-free solutions, J^{Cl}_{net} is significantly less than in normal Ringer's (−0.5±0.6 vs. 1.5±0.3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, $P < 0.05$). However, this change (−2.0 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) is equivalent to the change in J^R (−1.7 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) rather than the change in J^{Na}_{net} (−3.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$). Additionally, the decrease in I_{sc} (3.2 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) is roughly equal to the decrease in J^{Na}_{net} under these conditions. Thus, changes in Cl transport appear to be linked to changes in residual ion flux while changes in Na absorption are reflected by changes in I_{sc}.

We examined the effect of changing [Na] in the bathing media ($[Na]_0$) on I_{sc}. In these experiments, chloride was maintained at 121 mM with choline substituted for Na (Fig. 1). There is a definite, concentration-dependent effect of $[Na]_0$ on I_{sc}. Interestingly, there is minimal, if any, saturation until $[Na]_0$ reaches 100 mM. These studies establish that I_{sc} in cecum is a function of $[Na]_0$.

To further explore the link between J^{Na}_{net} and I_{sc}, we examined the relation between J^{Na}_{net} and I_{sc} of individual experiments in the various Ringer's solutions (Fig. 2). It is readily apparent that there is a strong correlation between J^{Na}_{net} and I_{sc} as Na absorption varies over a wide range of values. Therefore, it appears that Na absorption in the cecum is electrogenic.

Effect of amiloride. The diuretic amiloride inhibits electrogenic Na absorption in a wide variety of epithelia including

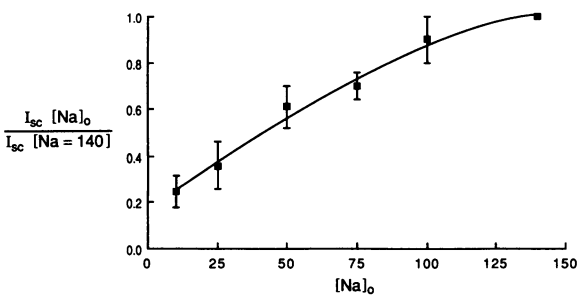

Figure 1. I_{sc} response to increasing $[Na]_0$. As [Na] in the fluid bathing rabbit cecum is increased from 10 to 140 (*x*-axis), there is a corresponding increase in I_{sc}. $I_{sc}[Na_0]/I_{sc}[Na_0 = 140]$ represents the ratio of current at a particular $[Na]_0$ to pair-matched controls exposed to 140 mM [Na]. *n*[Na = 10] 4; [Na = 25] 4; [Na = 50] 6; [Na = 75] 4; [Na = 100] 3; [Na = 140] 12.

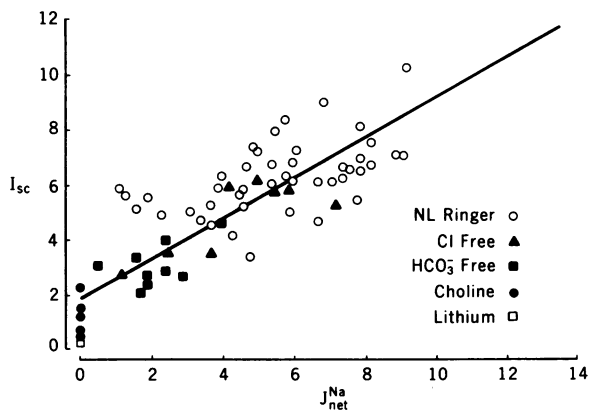

Figure 2. Correlation between J_{net} and I_{sc}. There is a strong linear correlation ($r = 0.8333$) between rates of Na absorption and I_{sc} as J^{Na}_{net} increases from 0 to 10 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ under a variety of conditions ($y = 0.76\,x + 1.96$). The data point for lithium represents four superimposed experiments. NL, normal.

distal colon. The effect of amiloride (10^{-4} M) added to the mucosal reservoir was examined in rabbit cecum (Fig. 3). In both normal and Cl-free Ringer's, amiloride did not alter I_{sc}. After exposure to amiloride, both unidirectional fluxes of Na increased slightly (J^{Na}_{m-s} from 7.6±0.5 to 8.1±0.6 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, P = NS; J^{Na}_{s-m} from 3.6±0.4 to 4.8±0.7 (P = NS). J^{Na}_{net} decreased insignificantly because of the larger increase in J^{Na}_{s-m}. In Cl-free Ringer's, J^{Na}_{net} was unchanged after exposure to amiloride although both unidirectional fluxes increased. Additionally, 10^{-3} M amiloride did not have a major effect on I_{sc} (data not shown). Thus, in cecum, amiloride fails to block the absorptive flux of Na and does not alter I_{sc}, implying a fundamentally different mechanism of Na absorption than that found in distal colon.

Phenamil inhibition of I_{sc} and J^{Na}. The amiloride analogue phenamil inhibits electrogenic Na transport but at a substantially lower K_i than the parent compound in toad bladder (11). In contrast to amiloride, phenamil significantly decreased both I_{sc} and J^{Na}_{net} in cecum. The dose-response curve (Fig. 4) indicates that 10^{-7} and 10^{-6} M phenamil have no effect on electrical parameters; at 10^{-5} M, I_{sc} is inhibited by 19% and at 10^{-4} M, the current is blocked by 81%. Neither pair-matched control tissues nor ceca exposed to amiloride exhibited changes in I_{sc}. There is a corresponding reduction in conductance of 1.5 $mS \cdot cm^{-2}$ in immediate response to phenamil, suggesting concomitant inhibition of a conductive process. Compared with the amiloride response in distal colon, the phenamil-induced decrease in I_{sc} is relatively slow; the nadir of I_{sc} is reached in ~ 15 min. Additionally, we examined the effect of the loop diuretic furosemide, which blocks the coupled Na-Cl (or Na-K-2Cl) cotransporter in a variety of epithelia. Furosemide did not have a significant effect on electrical parameters.

In well-defined systems of electrogenic transport, phenamil is ~ 20 times more potent than amiloride with a K_i in the range of 1.7×10^{-8} M. In the cecum, phenamil's K_i for electrogenic Na transport is ~ 5×10^{-5} M. Thus, cecal Na transport is at least two orders of magnitude less sensitive to phenamil.

In toad bladder, phenamil irreversibly inhibits Na transport (11). In cecum, changing the bathing solution partially reverses the effect of phenamil (Fig. 4). 50% of the phenamil-induced inhibition is irreversible, an effect similar to that seen in toad bladder. Thus, the failure of amiloride to block Na

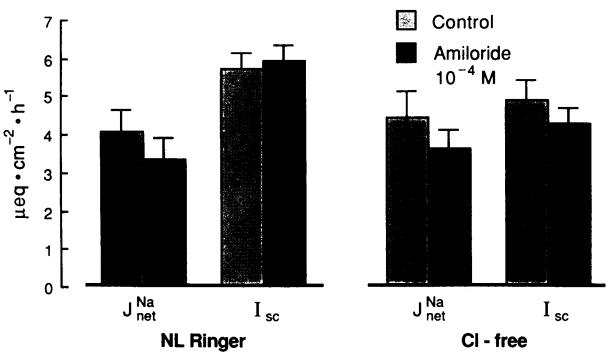

Figure 3. Amiloride effects on J_{net} and I_{sc}. Amiloride (10^{-4} M) added to the mucosal solution failed to inhibit J^{Na}_{net} or I_{sc} in either normal (NL) Ringer's or Cl-free solutions. There are no statistically significant differences (n = 8).

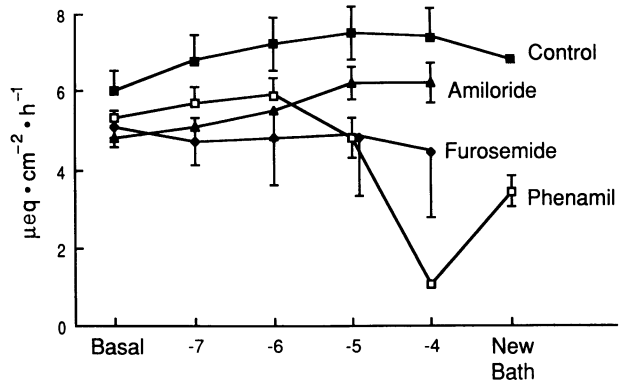

Figure 4. Dose–response curve for phenamil, amiloride, and furosemide. After tissue stabilization, the test drug was added in increasing concentration to the mucosal reservoir. Molar concentrations are plotted on the x-axis. In control and phenamil-treated tissues, the mucosal fluid was changed twice to test the reversibility of the drug's effect. There were 12 control, 8 phenamil, 5 amiloride, and 3 furosemide experiments.

transport and the high concentration of phenamil required to exert its effect both suggest that the amiloride-binding site for Na transport in the cecum has been substantially modified.

Amiloride has been shown to inhibit three distinct transport processes: electrogenic Na transport, Na-H exchange, and Na-Ca exchange. Amiloride analogues have been developed that differentially inhibit these three transport mechanisms. While phenamil has minimal effect on Na-H exchange ($K_i \geqq 1$ mM), it has reasonable activity on inhibiting Na-Ca exchange. To exclude the possibility that the observed effect of phenamil was via Na-Ca exchange, we tested three amiloride analogues that are primarily Na-Ca exchange inhibitors. The three agents (5-*N*-propyl-*N*-butyl)-2′4′-dichlorobenzamil, 5-[N[(4-chloro benzyl)-2′4′ dimethyl benzamil, and 2′4′-dimethyl benzamil) all failed to alter electrical parameters in the cecum (data not shown).

Flux studies were performed to confirm that the change in I_{sc} was due to a change in J^{Na}_{net}. In both the normal and chloride-free Ringer's, there was a strong correlation between the change in current and net Na flux (Table III). Both I_{sc} and J^{Na}_{net} were reduced by 4.5–5.0 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. In normal Ringer's, the decrease in J^{Na}_{net} was due primarily to a decrease in J^{Na}_{m-s}, consistent with inhibition of conductive Na entry. In chloride-free Ringer, the change in J^{Na}_{net} was due primarily to a change in J^{Na}_{s-m}. This pattern is similar to that seen in the series of experiments with amiloride in Cl-free Ringer. Under those conditions, J^{Na}_{s-m} increased from 2.2±0.2 to 3.4±0.6 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ (P = 0.005) with a corresponding increase in J^{Na}_{m-s}; there were, however, no significant changes in J^{Na}_{net} or I_{sc}. Thus, the increase in J^{Na}_{s-m} in Cl-free Ringer's is most likely due to associated conductance changes and independent of changes in I_{sc}. In this context, the trend towards decreased J^{Na}_{m-s} in response to phenamil may be interpreted as not inconsistent with the findings of phenamil's effect in normal Ringer's.

10^{-4} M phenamil does not block I_{sc} entirely, while it decreased J^{Na}_{net} to zero. This, along with the observation that there is a significant I_{sc} in choline Ringer's, suggests that there are additional electrogenic processes in cecum. The possible sources for this current are K absorption or HCO_3 secretion (see below).

Table III. Phenamil Inhibition of I_{sc} and J_{net}^{Na}

Group	J^{Na} m-s	J^{Na} s-m	J^{Na} net	I_{sc}	G_t
			$\mu eq \cdot cm^{-2} \cdot h^{-1}$		$mS \cdot cm^{-2}$
A. Normal Ringer's					
Control	8.3±0.7	4.6±0.5	3.6±0.9	6.64±0.50	6.6±0.8
Phenamil 10^{-4} M	5.6±0.3	6.6±0.8	−0.9±0.6	1.71±0.40	6.6±0.7
P	0.03	NS	0.01	0.01	NS
B. Chloride-free					
Control	7.8±1.3	3.9±0.7	3.9±1.6	5.49±1.10	4.4±0.5
Phenamil 10^{-4} M	6.7±0.5	7.9±0.8	−1.0±0.6	0.94±0.24	5.4±0.9
P	NS	.003	.02	.007	NS

Group A results are given for four animals. Phenamil was added to the mucosal chamber after the initial control flux. In a different series of rabbit ceca bathed with chloride-free Ringer's (group B), phenamil 10^{-4} M blocked J_{net}^{Na} (n = 5).

Modifying effects of sulfhydryl reagents. Pharmacologic agents that modify membrane sulfhydryl groups have previously been shown to alter the transport characteristics of amiloride-sensitive electrogenic Na transport in a variety of tight epithelia. Mercurial compounds such as PCMBS presumably form mercaptide bonds with exposed -SH groups in apical membranes (12). We therefore examined the effect of 10^{-3} PCMBS on the electrical parameters of cecal Na transport (Fig. 5).

PCMBS initiated a gradual increase in I_{sc} and G_t, reaching a plateau at ~ 15 min. Addition of phenamil caused a decrease of I_{sc} of 14.7 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, from 17.5 to 2.8 $\mu eq \cdot cm^{-2} \cdot h^{-1}$. This was accompanied by a decrease in conductance of 3 $mS \cdot cm^{-2}$. However, PCMBS added after phenamil had no significant effect on either I_{sc} or G_t. There was no difference in the post-phenamil electrical parameters, regardless of whether the tissue had been exposed to PCMBS or not. Thus, PCMBS significantly stimulates a phenamil-inhibitible increase in I_{sc}; pretreatment of the cecal epithelium with phenamil blocks the effect of PCMBS.

Potassium fluxes in rabbit cecum (Table IV). Steady-state fluxes in the cecum indicate that, under basal conditions, potassium is secreted. Addition of phenamil does not significantly alter K transport. Thus, potassium absorption is unlikely to be the source of the residual current either after addition of phenamil or in choline-free Ringer's.

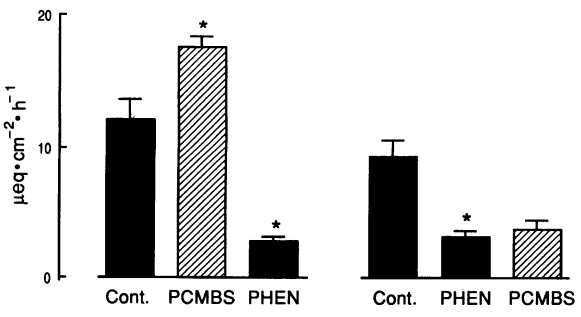

Figure 5. Effects of PCMBS and phenamil (*PHEN*) on I_{sc}. PCMBS (10^{-3} M) caused a significant increase in I_{sc}. Phenamil (10^{-4} M) reversed this effect and inhibited most of the basal I_{sc}. PCMBS added after phenamil did not increase I_{sc}. *P 0.05, n = 7 for each group.

Epinephrine stimulates Na absorption. Epinephrine, as an $\alpha 2$ adrenergic agonist, stimulates electrically neutral Na-Cl co transport (13, 14). In cecum, epinephrine 5.5 × 10^{-6} M added serosally stimulates electroneutral Na absorption (Table V). This occurs primarily through an increase in J_{m-s}^{Na}. The increase in J_{net}^{Cl} is not significant; J_{m-s}^{Cl} does not change, but J_{s-m}^{Cl} decreases from 7.6±0.8 to 6.4±0.7 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, P = 0.0507. Given the increased conductance and the trend over time for fluxes to increase, this probably represents a decrease in transcellular J_{s-m}^{Cl}. In bicarbonate-free Hepes-buffered solutions, epinephrine does not stimulate net Na absorption (control 2.1±0.3, post-epinephrine 2.0±0.7 $\mu eq \cdot cm^{-2} \cdot h^{-1}$, n = 8, P NS). Thus, there is a component of Na transport that is electroneutral, bicarbonate dependent, and epinephrine sensitive. It is not clearly coupled to Cl movement and is of lesser magnitude than that found in the proximal colon (1).

Response to secretagogues. The secretory response of cecum was assessed by examining the effect of 8BrcAMP, 8BrcGMP, and theophylline (Table VI). The three secretagogues elicited a similar pattern of response. I_{sc} rose by 1.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ after addition of 8BrcAMP, 8BrcGMP, and theophylline. There were no significant alterations in either unidirectional or net Na fluxes. However, Cl fluxes changed in a manner consistent with electrogenic Cl secretion: under all three conditions J_{s-m}^{Cl} increased. J_{m-s}^{Cl} either stayed the same (cGMP, theophylline) or increased modestly (cAMP); J_{net}^{Cl} decreased. The changes in Cl transport was of the same order of magnitude as the change in I_{sc}. Therefore, the cecum exhibits a modest capacity for electrogenic Cl secretion; secretory stimuli do not alter Na transport.

Bicarbonate transport. By employing a pH stat system, we measured the rates of luminal and serosal alkalinization (Fig. 6). This experimental design requires one side of the tissue to be bathed in HCO_3 Ringer's with the opposite side exposed to a HCO_3-free solution. Under these conditions, it is readily apparent that I_{sc} was significantly higher in the absence of mucosal HCO_3 (5.19±0.58 $\mu eq \cdot cm^{-2} \cdot h^{-1}$) than serosal HCO_3 (3.01±0.25 $\mu eq \cdot cm^{-2}$) P = 0.003 (Fig. 4). This suggests that the changes seen in I_{sc} and J_{net}^{Na} in HCO_3-free solutions (Table I)

Table IV. Potassium Transport in Cecum

	J^K			I_{sc}	G_t
	m-s	s-m	net		
	$\mu eq \cdot cm^{-2} \cdot h^{-1}$				$mS \cdot cm^{-2}$
Control (5)	0.20±0.05	0.73±0.10	−0.52±0.09	7.65±0.74	6.2±0.7
Phenamil (4)	0.15±0.04	0.65±0.06	−0.40±0.13	1.44±0.14*	6.5±0.5

Number of animals is given in parentheses. An initial flux period measured basal K fluxes in cecum. A subsequent flux period assessed the changes in K transport after the addition of phenamil. Resistance matches were lost in one experiment during the second period flux. * $P < 0.05$ vs. control.

are due to the absence of serosal HCO_3 and are not related to inhibition of a coupled Na-HCO_3 entry across the apical membrane. Although $J^{HCO_3}_{m\text{-}s}$ and $J^{HCO_3}_{s\text{-}m}$ were roughly equivalent, suggesting that, under basal conditions, a minimal amount of transcellular HCO_3 transport occurs, calculation of net HCO_3 transport from these data is not warranted because the flux measurements were obtained with imposed chemical gradients that differentially altered the electrical parameters and ion transport characteristics of the epithelium.

In response to epinephrine, $J^{HCO_3}_{m\text{-}s}$ increases from 1.8±0.7 to 3.2±0.7 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ ($P = 0.022$) while $J^{HCO_3}_{s\text{-}m}$ remains essentially unchanged (1.3±0.6 before; 1.4±0.5 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ post-epinephrine (P NS). This suggests that epinephrine elicits active HCO_3 absorption in rabbit cecum in addition to its effects on Na transport.

Discussion

Recent studies have clearly established that there is a significant segmental heterogeneity of ion transport properties in the colon. Whereas Na absorption in the distal colon is electrogenic and inhibited by low-dose amiloride, Na transport in proximal colon is electroneutral and unaffected by low-dose amiloride (1). It is becoming increasingly apparent that there are variations within the rabbit proximal colon; the 3F (triple-haustrated) segment within 10 cm of the proximal colon exhibits different transport parameters from the 1F (single-haustrated) segment of more distal (beyond 10 cm) proximal colon. Our observations indicate that the cecum is significantly different from the proximal colon and possesses a distinct mechanism for Na absorption.

Basal electrical measurements reveal that the rabbit cecum exhibits a strikingly higher basal I_{sc} in comparison with other intestinal segments. The I_{sc} of 6 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ is roughly double that found in ileum or distal colon; in fact, it is in the range found in glucose-stimulated ileum or steroid-treated distal colon (9, 15). Although studies examining cecal ion transport are limited, both Clauss et al. (4) (in the rabbit) and Will et al. (16) (in the rat) have found the highest I_{sc} within the intestine in the cecum. However Loeschke et al. (17), studying the effects of small bowel resection found cecal PD lower than that of colon.

Cecum absorbs Na efficiently. Several lines of evidence suggest that this Na absorption is electrogenic. There is a strong correlation between J^{Na}_{net} and I_{sc} (Fig. 2). As [Na] in the bathing media increases, there is a proportionate rise in I_{sc}. The increase is linear and begins to saturate at 100 mM $[Na]_o$. Maneuvers that cause a change in J^{Na}_{net} are accompanied by equivalent changes in I_{sc}. For example, Na replacement decreases both I_{sc} and J^{Na}_{net} proportionately. Anion replacement causes similar changes in both J^{Na}_{net} and I_{sc}. Most importantly, phenamil has an equivalent effect on I_{sc} and J^{Na}_{net}. The equivalence of J^{Na}_{net} and I_{sc} under a wide variety of conditions suggests that Na absorption is electrogenic.

However, the mechanism of cecal Na transport is not typical of "classic" epithelial electrogenic Na absorption, such as found in distal colon. Inhibition of Na absorption by low-dose (10^{-4} or 10^{-5} M) amiloride has almost become an operative definition of electrogenic Na transport. In our studies, amiloride failed to block either Na absorption or I_{sc}. In distal colon, an equivalent dose of amiloride abolishes both J^{Na}_{net} and I_{sc}. Interestingly, Clauss et al. (4) have also observed a lack of effect of amiloride in rabbit cecum while Will et al. (16) were able to demonstrate only a 3% inhibition of I_{sc} by amiloride. Thus, unlike distal colon, electrogenic Na transport in cecum appears to be resistant to amiloride.

There are several additional factors that differentiate Na transport in the rabbit cecum from that in distal colon. I_{sc} and

Table V. Effects of Epinephrine in Cecum

	J^{Na}			J^{Cl}			I_{sc}	J^R	G_t
	m-s	s-m	net	m-s	s-m	net			
	$\mu eq \cdot cm^{-2} \cdot h^{-1}$								$mS \cdot cm^{-2}$
I Control	9.7±1.6	3.8±0.7	5.9±1.3	8.3±0.6	7.6±0.8	0.9±1.2	6.44±0.43	1.5±0.6	7.1±0.8
II Epinephrine	12.9±1.3	5.1±0.8	7.8±1.1	8.4±0.5	6.4±0.7	2.0±0.8	6.98±0.46	1.2±0.5	12.0±1.2
P	0.0001	0.043	0.019	NS	0.051	NS	NS	NS	0.001

Results are given for 10 animals. The protocol for these studies involved an initial basal flux period of 30 min (I control), addition of 5.5×10^{-6} M epinephrine to the serosal reservoir during a 20-min stabilization period and a subsequent 40-min flux (II Epinephrine).

Table VI. Electrogenic Cl Secretion in Cecum

	J^{Na}			J^{Cl}					
	m-s	s-m	net	m-s	s-m	net	I_{sc}	J^R	G_t
	$\mu eq \cdot cm^{-2} \cdot h^{-1}$								$mS \cdot cm^{-2}$
I. Theophylline									
Control	10.2±0.6	4.8±0.6	5.4±0.6	10.7±0.3	8.4±0.5	2.3±0.7	6.47±0.33	3.4±0.6	7.8±0.6
Theophylline (5)	10.9±0.8	5.0±0.7	5.9±0.7	9.8±0.6	9.9±0.8	−0.1±0.6	8.13±0.44	2.2±0.6	11.3±1.15
P	NS	NS	NS	NS	0.0397	NS	0.0011	NS	NS
II. 8BrcAMP									
Control	9.0±0.7	3.8±0.7	5.2±0.4	8.0±0.7	6.0±0.9	2.0±0.5	6.10±0.3	2.9±0.6	6.5±0.4
cAMP (8)	9.8±1.3	4.3±0.7	5.5±0.9	9.8±1.3	8.9±1.1	0.9±0.6	7.41±0.5	2.8±0.6	9.6±1.7
P	NS	NS	NS	0.0496	0.0055	0.0085	0.0047	NS	NS
III. 8BrcGMP									
Control	10.3±0.6	2.5±0.4	7.8±0.4	10.7±0.9	7.8±1.0	2.9±1.0	6.57±0.20	1.6±1.1	6.3±0.3
cGMP (8)	11.8±1.2	3.7±0.6	8.1±1.2	10.1±1.0	8.7±0.7	1.4±1.0	8.50±0.50	1.9±0.7	8.7±1.1
P	NS	NS	NS	NS	NS	0.0279	0.0022	NS	0.0327

The number of animals for each group is given in parentheses. The protocol for these experiments consisted of a control flux, addition of secretagogue to the serosal reservoir (10^{-3} M theophylline, 10^{-4} BrcAMP, 10^{-4} 8BrcGMP), and a second flux period.

J^{Na}_{net} are clearly related to external Na ($[Na]_o$) in both distal colon and cecum; however, in distal colon I_{sc} and J^{Na}_{net} appear to "saturate" at a much lower $[Na]_o$ (40–60 mM) than in cecum, suggesting that, on a macroscopic basis at least, the kinetics of transport are different.

The response to anion substitution also differentiates cecum and distal colon. In distal colon, Cl replacement with a series of impermeant anions results in a rise in I_{sc} and enhancement of J^{Na}_{net} (18). In cecum, chloride replacement with sulfate causes a modest decrease in both I_{sc} and J^{Na}_{net}. While HCO_3 has previously been shown to stimulate neutral Na absorption in the gallbladder (19), it has not, to our knowledge, been demonstrated to have a significant effect on electrogenic Na transport.

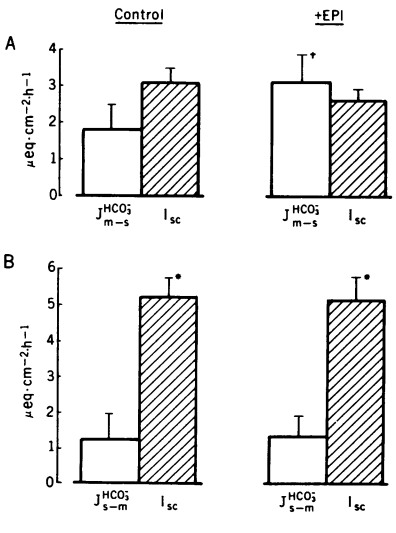

Figure 6. Bicarbonate transport in cecum. (*A*) $J^{HCO_3}_{m-s}$ increases significantly after exposure to 5.5 μM epinephrine without a change in I_{sc}. (*B*) $J^{HCO_3}_{s-m}$ is unchanged by an identical dose of epinephrine. The I_{sc} in ceca exposed to a bicarbonate-free serosal solution (*A*) exhibit a significantly lower I_{sc} than pair-matched tissues in which serosal HCO_3 was present (*B*). See text for details of experimental protocol. †*P* 0.05 vs. control $J^{HCO_3}_{m-s}$. **P* 0.005 vs. corresponding I_{sc} in A.

Phenamil inhibits electrogenic Na transport. Phenamil clearly inhibits both I_{sc} and J^{Na}_{net}. This observation provides an important insight into the mechanisms of electrogenic Na absorption in the cecum. The present data are most consistent with significant alterations of the binding sites associated with the classical Na channel rather than a fundamentally distinct channel. Work by several investigators studying electrogenic Na absorption in a variety of tight epithelia has suggested that there are specific loci associated with the Na channel that modify its behavior: an amiloride-binding site (20), a Na self-inhibition site (21, 22), a sulfhydryl group sensitive to mercurial agents (12), a trypsin-sensitive site (23), and a methyl receptor locus (24). There may be overlap among these sites; however, it appears that the amiloride-binding and Na self-inhibition sites are distinct (20).

Although amiloride has no effect on electrogenic Na absorption in cecum, phenamil does inhibit both I_{sc} and J^{Na}_{net}. Compared with its effect in other epithelia, phenamil in cecum acts only in high doses. These observations suggest that the interaction between the amiloride analogues and a specific binding site associated with the channel are much weaker in cecum than in other epithelia.

Lindemann has described the phenomenon of Na self-inhibition, in which increasing [Na] bathing the apical membrane of tight epithelia results in closure of Na channels (21, 22). Presumably Na^+ ions react with specific modifying sites located in the vicinity of the Na-conductive channel; the channel closes when the site is occupied by Na. This leads to the typical type of saturation kinetics associated with Na absorption in the distal colon. In cecum, however, there is no obvious "saturation"; the rise in I_{sc} parallels the increase in [Na] to 100 mM.

This is consistent with a lack of self-inhibition, which may be due to an absent or ineffective self-inhibition site.

Sulfhydryl reagents alter electrogenic Na transport in a variety of epithelia. In rabbit distal colon, PCMBS "freezes" the Na conductance of the apical membrane at a mean value of 2–3 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ and renders the transport amiloride-insensitive (25). Sulfhydryl reagents also stimulate electrogenic Na absorption in frog skin (12, 26); however, in contrast to distal colon, this Na transport is blocked by amiloride. The response of the cecum to PCMBS and phenamil is similar to that found in frog skin rather than distal colon: i.e., sulfhydryl reagent stimulation of Na transport that retains its sensitivity to an amiloride analogue. Thus, the Na transport system in rabbit cecum is associated with sulfhydryl groups that can be modified by mercurial agents.

Although there is considerable evidence for in vitro pharmacologic modification of Na channels, there is also some precedent for in vivo alterations of this transport system (27, 28). Along these lines, Leng-Peschlow and Marty (29) have observed that, in a surgically created cecal pouch, pretreatment of the pouch with cecal contents over several days resulted in net absorption of Na and water. In contrast, pouches exposed only to electrolyte solutions secreted Na and water. Considering the degree of stasis normally found in the rabbit cecum, it is conceivable that there may be a bacterial enzymatic effect on the epithelium. Our in vitro data are consistent with an electrogenic Na transport system (i.e., channel) in which the modifying effects of Na self-inhibition and amiloride binding have been altered.

Effect of epinephrine. There is little evidence for electroneutral Na absorption under basal conditions in cecum. However, epinephrine stimulates Na absorption (from 5.9±1.3 to 7.8±1.1 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ with little change in I_{sc}. Although $J^{Cl}_{s\text{-}m}$ decreases significantly, there is no increase in the absorptive flux of Cl. However, as the pH stat data indicates, epinephrine elicits an increase in $J^{HCO_3}_{m\text{-}s}$ from 1.8±0.7 to 3.2±0.7 $\mu eq \cdot cm^{-2} \cdot h^{-1}$ without a change in $J^{HCO_3}_{s\text{-}m}$. Thus epinephrine-stimulated Na transport in the cecum is accompanied by changes in HCO_3 fluxes. Similar results have been reported by Smith and Sullivan (5, 30) in both ileum and the proximal portion of the proximal colon. We also observed that epinephrine-stimulated Na absorption did not occur in HCO_3-free solutions. Electroneutral Na transport in the cecum, therefore, appears to be linked to HCO_3. Compared with the amount of electrogenic Na transport, electroneutral Na absorption is a relatively minor component of overall Na absorption in the cecum.

Anion transport, electrogenic secretion, and residual current. A modest rate of basal Cl absorption was present in the cecum. Na substitution with choline did not significantly alter Cl absorption; ceca bathed in HCO_3-free fluid exhibited a modest reversal to Cl secretion. With an imposed transepithelial HCO_3 gradient during the pH stat experiments, the cecum demonstrated bidirectional HCO_3 transport.[2]

Both cAMP- and cGMP-mediated secretagogues elicited a response consistent with electrogenic Cl secretion. The change in I_{sc} we demonstrated is similar to that found by Rao et al. (31). (No flux measurements were performed in that latter study.) The flux studies do not indicate inhibition of neutral Na-Cl cotransport. The rabbit cecum, then, is analogous to the proximal colon: under basal conditions, electroneutral Na absorption is nil; secretagogues do not alter basal Na absorption; but epinephrine can elicit electroneutral Na absorption. However, unlike proximal colon, the cecum has the capacity for electrogenic Cl secretion.

Although Na absorption in the cecum is electrogenic, there is substantial evidence that there are additional electrogenic transport processes operative in this epithelium. In Na-free choline Ringer's, a significant current remains. In normal Ringer's, phenamil blocks Na absorption but does not eliminate I_{sc} entirely. Flux studies indicate that neither chloride nor potassium fluxes can account for the residual current. The situation with HCO_3 is complex since "net fluxes" can not be calculated (see above). However, it is possible, given the post-phenamil current in Cl-free Ringer's, that there is a component of HCO_3 secretion.

The cecum in context. It has become increasingly apparent that the gut, much like the nephron, has elaborate segmental variations. The rabbit cecum is voluminous; it comprises 50% of the macrosurface area of the colon and therefore is likely to have a major effect on colonic ion transport. Additionally, because of cecal stasis, the contact time of the luminal fluid with the epithelium is prolonged in comparison with the remainder of the gut, potentiating its role in intestinal fluid balance.

Prior in vivo studies have suggested that the bulk of colonic fluid is absorbed proximally rather than distally. However, we had previously shown that basal electrolyte absorption in the rabbit proximal colon is insignificant (1). The finding that the cecum has a major absorptive capacity for Na may help, in part, to resolve these discrepancies. Additionally, although the cecum does respond to secretagogues with electrogenic Cl secretion, Na absorption is unaltered and the total osmolar flux remains absorptive. Thus, in the face of secretory stimuli, the cecum may retain the ability to conserve fluid.

The major transport pathway in the cecum is electrogenic Na absorption that is amiloride insensitive, but blocked by phenamil. There is some precedent for such an absorptive mechanism. In ileum there appears to be a component of Na absorption that is electrogenic and not coupled to nutrient absorption (32). Additionally, recent studies in human colon in vitro have suggested that there is chloride-independent, amiloride-insensitive electrogenic Na absorption (33). These pathways have obvious biological and physiological importance; their regulation will clearly require further study.

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