Ion Transport in Proximal Colon of the Rat

Sodium Depletion Stimulates Neutral Sodium Chloride Absorption

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Abstract

The model of sodium and chloride transport proposed for the colon is based on studies performed in the distal segment and tacitly assumes that ion transport is similar throughout the colon. In rat distal colon, neutral sodium-chloride absorption accounts for the major fraction of overall sodium absorption and aldosterone stimulates electrogenic, amiloride-sensitive sodium absorption. Since we have demonstrated qualitative differences in potassium transport in proximal and distal segments of rat colon, unidirectional ^{22}Na and ^{36}Cl fluxes were performed under short-circuit conditions across isolated proximal colon of control and sodium-depleted rats with secondary hyperaldosteronism. In the control group, net sodium absorption (J_{net}^{Na}) (7.4±0.5 μeq/h · cm²) was greater than I_{sc} (1.4±0.1 μeq/h · cm²), and J_{net}^{Cl} was 0 in Ringer solution. Residual flux (J^R) was −5.2±0.5 μeq/h · cm² consistent with hydrogen ion secretion suggesting that neutral sodium absorption may represent sodium–hydrogen exchange. 1 mM mucosal amiloride, which inhibits sodium–hydrogen exchange in other epithelia, produced comparable decreases in J_{net}^{Na} and J^R (4.1±0.6 and 3.2±0.6 μeq/h · cm², respectively) without a parallel fall in I_{sc}. Sodium depletion stimulated J_{net}^{Na}, J_{net}^{Cl}, and I_{sc} by 7.0±1.4, 6.3±1.9, and 0.8±0.2 μeq/h · cm², respectively, and 1 mM amiloride markedly inhibited J_{net}^{Na} and J_{net}^{Cl} by 6.0±1.1 and 4.0±1.6 μeq/h · cm², respectively, with only a minimal reduction in I_{sc}. Conclusions: the predominant neutral sodium-absorptive mechanism in proximal colon is sodium–hydrogen exchange. Sodium depletion stimulates electroneutral chloride-dependent sodium absorption (most likely as a result of increasing sodium–hydrogen and chloride–bicarbonate exchanges), not electrogenic chloride-independent sodium transport. The model of ion transport in the proximal colon is distinct from that of the distal colon.

Introduction

The mammalian colon absorbs sodium and chloride and secretes potassium and bicarbonate. Many investigations of colonic function have assumed that electrolyte transport processes are uniform throughout the large intestine. Thus, in vivo luminal perfusion experiments, in humans and experimental animals, have frequently been performed without regard to the possibility that electrolyte movement may differ in one or more segments (1–3). Further, in vitro studies of ion transport have until recently been primarily performed in the distal portion of the colon (4, 5). Recently, however, several reports have demonstrated that electrolyte transport is not identical in all portions of the mammalian large intestine and that the characteristics of both basal ion transport and the effect of aldosterone on sodium absorption may differ substantially in various colonic segments (5–10).

Electrolyte transport in the rat distal colon has been well characterized in a series of in vitro experiments performed in this laboratory (5, 11, 12). The model of ion transport that has evolved from these studies indicates that there are two modes of both sodium and chloride absorption: electrogenic sodium absorption, neutral sodium-chloride absorption and bicarbonate-dependent chloride absorption. Although potassium transport in the rat colon has been shown to exhibit markedly different characteristics in the proximal and distal segments (5, 9, 13), significant differences in basal sodium and chloride transport in segmental portions of the rat large intestine have not been described.

Recent in vivo studies from this laboratory have demonstrated that aldosterone increases the absorption of sodium by different mechanisms in the proximal and distal portions of the rat colon (10). In the distal segment, aldosterone stimulates electrogenic sodium transport by inducing amiloride-sensitive sodium channels in the apical membrane associated with a marked increase in the transmural potential difference. In contrast, in the proximal colon, whereas aldosterone also results in an increase in sodium absorption, chronic hyperaldosteronism does not induce amiloride sensitivity or alter transmural potential difference (10).

The present experiments were conducted to examine the mode of sodium and chloride absorption in the proximal colon under basal conditions and in sodium-depleted animals. The results demonstrate that in the basal state sodium–hydrogen exchange is the predominant sodium transport process. Sodium depletion stimulates neutral sodium-chloride absorption by a mechanism that most likely involves sodium–hydrogen and chloride–bicarbonate exchange processes. Therefore, sodium and chloride transport in the proximal colon of the rat has distinctive and unique characteristics.

Methods

Nonfasting male Sprague-Dawley rats weighing between 250 and 300 g were used in all experiments. Two groups of animals, a control and an experimental group, were studied. Control rats were fed a standard Purina Chow diet (Ralston Purina Co., St. Louis, MO), containing 25 meq of Na/100 g of food. The experimental group, referred to as the sodium-depleted group, was fed a paste diet without added Na for 7–9 d to induce chronic secondary hyperaldosteronism, as previously reported by our laboratory (5). The mean plasma aldosterone level in a comparable group of experimental animals was 473±66 vs. 4.6±0.7 ng/dl in controls (14). All animals were given tap water to drink ad libitum.

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After killing the animals, we obtained two pieces of colonic mucosa from each animal from the segment immediately distal to the cecal–colonic junction; the epithelium studied thus represented the most proximal portion of the colon. The colonic mucosa, stripped of the serosa and part of the muscle layers, was mounted in Lucite chambers (surface area 1.13 cm^2), bathed on both sides with equal volumes of Ringer's solution at 37°C and continuously oxygenated with 95% O_2/5% CO_2. The Ringer's solution contained (in millimoles per liter): Na, 140; K, 5.2; Ca, 1.2; Mg, 1.2; Cl, 119.8; HCO_3, 25; HPO_4, 2.4; H_2PO_4, 0.4; and glucose, 10 (pH, 7.4). Sodium-free Ringer's solution was prepared by equimolar replacement of sodium with choline and chloride-free Ringer's solution by equimolar replacement with isethionate. The bicarbonate-free Ringer solution was also prepared by equimolar replacement with isethionate. Transepithelial potential difference (PD)[1] and short-circuit current (I_{sc}) were measured, and conductance (G) was calculated by methods previously described (5, 9).

Unidirectional transmural sodium and chloride fluxes were performed with ^{22}Na and ^{36}Cl under short-circuit conditions as described in previous studies (5). Tissues were paired on the basis of a conductance difference of <10%. Net ion transport (J_{net}) is defined as the difference between mucosal-to-serosal transport (J_{ms}) and serosal-to-mucosal transport (J_{sm}). Because these studies were performed under short-circuit conditions, net values represent active ion transport: positive values reflect active absorption and negative values active secretion. The residual flux (J^R) was calculated by the equation: $J^R = I_{sc} - (J^{Na}_{net} - J^{Cl}_{net})$.

Because previous studies have established a constant rate of ^{22}Na and ^{36}Cl occurred within 14 min after tracer addition (5), unidirectional flux measurements were initiated at least 14 min after the addition of ^{22}Na and ^{36}Cl. Preliminary experiments also demonstrated that the rate of sodium and chloride transport remained constant over four 15-min flux periods. Only in the sodium-depleted animals was there a gradual though significant decline in I_{sc} during the 60 min of flux determinations. The stability of ion transport permitted an experimental design in which each tissue served as its own control. 3 min after the conclusion of the two basal flux periods, potential modifiers of ion transport were added to either the serosal or mucosal bathing solution. After a 12-min equilibration period, an additional 15-min flux period was performed. The effect of amiloride, acetazolamide, bumetanide, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid (SITS), 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), or theophylline on ion transport was evaluated by comparing the period immediately after the addition of the agent with the basal periods immediately prior to its addition. 5 mM theophylline was added to the serosal bathing solution. 1 mM amiloride and 1 mM bumetanide were added to the mucosal bathing solution, whereas 0.1 mM acetazolamide was added to both mucosal and serosal bathing solutions. 1.0 mM SITS was added to either mucosal or serosal bathing solutions; 2.0 mM DIDS was added to the mucosal bathing solution.

Materials. ^{36}Cl was obtained from New England Nuclear (Boston, MA), ^{22}Na from New England Nuclear and Amersham-Searle (Arlington Heights, IL), acetazolamide from American Cyanamide Co. (Pearl River, NY), amiloride from Merck, Sharp & Dohme Research Laboratories (West Point, PA), theophylline, SITS, and DIDS from Sigma Chemical Co. (St. Louis, MO), and bumetanide from Hoffmann-La Roche, Inc. (Nutley, NJ).

Statistics. All values are expressed as the mean±standard error of the mean. Statistical analysis was performed using the paired and unpaired Student's t test. A P value of <0.05 was considered significant (15).

Results

Studies in the control group. Table I shows unidirectional and net sodium and chloride fluxes in Ringer solution across proximal colonic mucosa of the control group. Net sodium absorption, indicative of active transport, was present although net chloride transport was not different from 0. In that net sodium absorption (7.4±0.5 μeq/h·cm^2) was significantly greater than I_{sc} (1.4±0.1 μeq/h·cm^2, $P < 0.001$), these data demonstrate that neutral absorption, defined as the difference between J^{Na}_{net} and I_{sc}, is present in the rat proximal colon. The J^R was −5.2±0.5 μeq/h·cm^2 and is consistent with either cation (potassium or hydrogen) secretion or anion (bicarbonate) absorption. Because the rate of net potassium secretion in this tissue has been shown to represent <10% of J^R (9), it is unlikely that potassium secretion accounts for J^R. Additional experiments were performed, therefore, to determine whether the neutral sodium absorptive process represented sodium–hydrogen exchange.

Because high concentrations of amiloride inhibit sodium–hydrogen exchange in many epithelia (16), the effect of 1 mM mucosal amiloride on sodium transport was evaluated (Table II). Amiloride had no effect on I_{sc} (1.4±0.2 vs. 1.2±0.1 μeq/h·cm^2) but decreased both J^{Na}_{ms} and J^{Na}_{net} by 4.2±0.7 μeq/h·cm^2 and 4.1±0.6 μeq/h·cm^2, respectively. Amiloride produced an equivalent fall in J^R (3.2±0.6 μeq/h·cm^2). Amiloride did not alter unidirectional or net chloride movement. These data are consistent with the presence of sodium–hydrogen exchange in the proximal colon under basal conditions.

To determine the ionic dependence of sodium and chloride transport in the proximal colon, fluxes of both ions were studied in ion-substitution experiments (Table I). In sodium-free Ringer's solution I_{sc} was significantly reduced to 0.2±0.1 μeq/h·cm^2 ($P < 0.001$) and J^R was decreased to 0.3±0.5 μeq/h·cm^2 ($P < 0.001$). Unidirectional and net chloride fluxes were unchanged compared to those in Ringer's solution. Removal of chloride from the bathing solution, however, significantly reduced net sodium absorption by 6.3±0.7 μeq/h·cm^2 ($P < 0.001$) primarily as a result of a decrease in J^{Na}_{ms} of 4.7±0.9 μeq/h·cm^2 ($P < 0.001$) (Table I). I_{sc} was also decreased in chloride-free Ringer solution compared to Ringer solution, whereas the conductance in both sodium-free and chloride-free Ringer's solutions was similar to that observed in Ringer's solution.

Sodium and chloride fluxes were also performed in the absence of bicarbonate. J^{Na}_{net} was markedly reduced in the bicarbonate-free Ringer's solution (1.5±0.4 vs. 7.4±0.5 μeq/h·cm^2, $P < 0.001$) primarily as a result of a decrease in J^{Na}_{ms} (7.0±0.5 vs. 14.8±0.5 μeq/h·cm^2, $P < 0.001$). There was no significant change in either J^{Cl}_{net} or I_{sc}, but J^R was inhibited (−1.1±1.1 vs. −5.2±0.5 μeq/h·cm^2, $P < 0.01$). In the absence of bicarbonate J^{Na}_{net} (1.5±0.4 μeq/h·cm^2) was equivalent to I_{sc} (1.8±0.2 μeq/h·cm^2). Acetazolamide (0.1 mM) did not alter either J^{Na}_{net} or J^{Cl}_{net} in either Ringer's (Table III) or HCO_3^--free Ringer solutions (data not shown). Neither J^{Na}_{net} nor J^{Cl}_{net} were inhibited in Ringer's solution by either mucosal or serosal addition of 1.0 mM SITS (data not shown).

Because in the rat distal colon theophylline inhibits neutral sodium-chloride absorption and stimulates active chloride secretion, which is reflected by a rise in I_{sc} (17), we also explored the effect of this agent on sodium and chloride transport in the proximal segment. Addition of 5 mM theophylline to the serosal solution (Table IV) reduced net sodium absorption by 3.4±0.8 μeq/h·cm^2. Theophylline increased I_{sc} and induced net chloride secretion (−3.3±1.0 μeq/h·cm^2). In that the absolute rise in J^{Cl}_{sm} (3.0±0.7 μeq/h·cm^2), J^{Cl}_{net} (−2.8±0.9 μeq/h·cm^2) and I_{sc} (2.2±0.2 μeq/h·cm^2) were equivalent, it seems likely that the major effect of theophylline on chloride transport involved

1. *Abbreviations used in this paper:* DIDS, 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid; G, conductance; I_{sc}, short-circuit current; J_{ms}, mucosal-to-serosal transport; J_{net}, net transport; J^R, residual flux; J_{sm}, serosal-to-mucosal transport; PD, potential difference; SITS, 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid.

*Table I. Unidirectional and Net Sodium and Chloride Fluxes across Proximal Colonic Mucosa of Control and Sodium-depleted Animals in Ringer's Solution, and in Sodium-free, Chloride-free, and Bicarbonate-free Ringer's Solutions**

		Na flux			Cl flux						
Solution	n	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	J^R
		$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	$\mu eq/h \cdot cm^2$	mV	mS/cm^2	$\mu eq/h \cdot cm^2$
Ringer's											
Control	40	14.8 ±0.5	7.4 ±0.2	7.4 ±0.5	10.7 ±0.7	9.8 ±0.3	0.9 ±0.6	1.4 ±0.1	2.3 ±0.2	16.7 ±0.6	−5.2 ±0.5
Experimental group	18	25.6 ±1.2	11.1 ±0.5	14.5 ±1.3	24.6 ±1.8	17.3 ±0.7	7.2 ±1.8	2.2 ±0.2	2.9 ±0.4	21.7 ±1.4	−5.0 ±1.0
P*		<0.001	<0.02	<0.001	<0.001	<0.001	<0.001	<0.001	<0.02	<0.01	NS
Chloride-free Ringer's											
Control	8	10.1 ±0.8	9.0 ±1.1	1.1 ±0.4	—	—	—	0.4 ±0.3	0.9 ±0.7	15.1 ±1.4	−0.7 ±0.3
Experimental group	5	11.0 ±0.8	9.3 ±0.7	1.8 ±0.5	—	—	—	0.8 ±0.4	1.3 ±0.6	15.2 ±0.8	−1.0 ±0.4
P*		NS	NS	NS				NS	NS	NS	NS
Sodium-free Ringer's											
Control	6	—	—	—	9.7 ±0.9	9.8 ±0.8	−0.1 ±0.5	0.2 ±0.1	0.5 ±0.2	13.9 ±1.7	0.3 ±0.5
Experimental group	8	—	—	—	7.4 ±0.6	7.5 ±0.5	−0.1 ±0.3	0.1 ±0.1	0.1 ±0.1	10.1 ±1.2	0.2 ±0.3
P*					<0.05	<0.05	NS	NS	<0.05	NS	NS
Bicarbonate-free Ringer's											
Control	9	7.0 ±0.5	5.5 ±0.4	1.5 ±0.4	5.9 ±0.6	7.3 ±0.9	−1.4 ±1.0	1.8 ±0.2	3.9 ±0.3	12.3 ±0.6	−1.1 ±1.1
Experimental group	8	10.5 ±0.7	7.1 ±0.4	3.4 ±0.7	14.7 ±2.5	17.5 ±1.9	−2.8 ±1.9	2.3 ±0.3	4.8 ±0.4	13.1 ±1.1	−3.8 ±1.6
P*		<0.02	<0.01	NS	<0.001	<0.001	NS	NS	NS	NS	NS

Mucosa from the proximal colon of two groups of animals were studied in Ringer's solution, and in Na-free, chloride-free, and bicarbonate-free Ringer's solutions. The control animals were fed a standard Purina Chow diet. The experimental group was maintained on a sodium-depleted diet to induce secondary hyperaldosteronism. Values represent the mean of two 15 min flux periods. Results are given as mean±SEM. Positive values represent net absorption, and negative values represent net secretion. n is the number of tissue pairs. * Compared to control group.

Table II. Effect of 1 mM Amiloride on Unidirectional and Net Na and Cl Fluxes in Control and Sodium-depleted Animals

	Na flux			Cl flux						
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	J^R
	$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$	mV	mS/cm^2	$\mu eq/h \cdot cm^2$
Control										
Period I	13.2	5.7	7.5	9.6	9.4	0.2	1.4	2.9	13.0	−5.9
	±0.7	±0.2	±0.8	±1.6	±1.2	±0.9	±0.2	±0.3	±1.1	±0.7
Period II	9.0	5.6	3.4	8.3	8.8	−0.5	1.2	2.4	13.4	−2.7
	±0.4	±0.3	±0.5	±1.1	±0.8	±0.6	±0.1	±0.2	±1.1	±0.5
P*	<0.001	NS	<0.001	NS	NS	NS	NS	NS	NS	<0.001
Sodium-depletion										
Period I	23.8	11.6	12.2	23.9	15.8	8.1	1.4	1.6	21.8	−2.8
	±1.6	±0.8	±1.8	±3.1	±1.0	±3.4	±0.2	±0.2	±1.3	±1.8
Period II	16.7	10.4	6.2	20.2	16.1	4.1	1.1	1.3	21.6	−1.1
	±1.3	±0.9	±1.4	±1.7	±0.6	±2.0	±0.2	±0.2	±1.2	±1.2
P*	<0.005	<0.02	<0.005	NS	NS	<0.05	<0.02	<0.05	NS	NS

Period I is the 15-min flux period before the addition of amiloride to the mucosal solution. 12 min after the addition of amiloride, a second 15-min flux period was performed. See legend to Table I for additional details of experiment. Nine and six tissue pairs were studied in the control and sodium-depleted groups, respectively. * Compared to period I.

stimulation of active chloride secretion, rather than inhibition of sodium-dependent chloride absorption.

Studies in the sodium-depleted group. Sodium and chloride transport was also determined in a series of experiments in sodium-depleted animals with secondary hyperaldosteronism. Compared to the control group (Table I), sodium depletion significantly stimulated net sodium absorption by 7.1±1.4 $\mu eq/h \cdot cm^2$ and induced net chloride absorption (7.2±1.8 $\mu eq/h \cdot cm^2$). Despite the marked increase in J^{Na}_{net}, sodium depletion produced only a small rise in I_{sc} (0.8±0.2 $\mu eq/h \cdot cm^2$). The difference between J^{Na}_{net} and I_{sc} (6.2±1.4 $\mu eq/h \cdot cm^2$) produced by sodium depletion, which provides a measure of neutral sodium absorption, was equivalent to the stimulation of J^{Cl}_{net} (6.3±1.9 $\mu eq/h \cdot cm^2$). Sodium depletion did not change J^R.

Table III. Effect of 0.1 mM Acetazolamide on Unidirectional and Net Sodium and Chloride Fluxes of Control and Sodium-depleted Animals

	Na flux			Cl flux						
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	J^R
	$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$	mV	mS/cm^2	$\mu eq/h \cdot cm^2$
Control										
Period I	14.4	7.8	6.6	10.4	9.7	0.6	1.2	1.9	17.4	−4.8
	±1.2	±0.3	±1.1	±1.0	±0.5	±1.0	±0.1	±0.2	±0.9	±1.1
Period II	14.0	8.2	5.7	9.7	10.2	−0.5	0.9	1.4	17.9	−5.2
	±1.0	±0.6	±0.8	±0.9	±0.8	±0.7	±0.2	±0.3	±0.9	±0.8
P*	NS	NS	NS	NS	NS	<0.05	<0.05	<0.01	<0.05	NS
Sodium-depletion										
Period I	23.5	9.6	13.9	23.0	14.4	8.6	2.8	3.4	22.9	−2.4
	±2.5	±0.4	±2.4	±3.3	±0.4	±3.1	±0.4	±0.4	±1.7	±1.1
Period II	17.5	8.9	8.6	21.1	14.2	6.9	2.2	2.5	24.8	0.5
	±1.2	±0.6	±1.3	±2.3	±0.5	±2.0	±0.4	±0.3	±2.9	±1.5
P*	<0.01	NS	<0.02	NS	NS	NS	<0.005	<0.005	NS	<0.05

Period I is the 15-min flux period before the addition of acetazolamide to both mucosal and serosal bathing solution. 12 min after the addition of acetazolamide, a second 15-min flux period was performed. See legend to Table I for additional details of experiments. Eight and seven tissue pairs were studied in the control and sodium-depleted groups, respectively. * Compared to period I.

Table IV. Effect of 5 mM Theophylline on Unidirectional and Net Sodium and Chloride Fluxes of Control Group

	Na flux			Cl flux						
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	J^R
	$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$	mV	mS/cm^2	$\mu eq/h \cdot cm^2$
Period I	16.1	7.4	8.7	10.0	10.5	−0.5	1.4	2.4	17.8	−7.7
	±1.2	±0.5	±1.2	±0.7	±0.8	±0.9	±0.2	±0.5	±1.6	±1.1
Period II	13.8	8.5	5.3	10.2	13.5	−3.3	3.6	5.2	20.3	−5.0
	±1.1	±0.6	±1.2	±0.7	±0.7	±1.0	±0.4	±0.9	±1.6	±1.0
P*	<0.005	<0.05	<0.005	NS	<0.005	<0.02	<0.001	<0.001	<0.001	<0.005

Period I is the 15-min flux period before the addition of theophylline to the serosal solution. 12 min after the addition of theophylline, a second 15-min flux period was performed. See legend to Table I for additional details of experiment. Nine tissue pairs were studied. * Compared to period I.

To determine whether the stimulation of net sodium and chloride absorption by sodium depletion represented neutral sodium-chloride absorption or was secondary to parallel but independent sodium and chloride transport processes sodium and chloride fluxes were measured in chloride-free and in sodium-free Ringer's solution, respectively (Table I). In the sodium-depleted group, removal of chloride from the bathing solution resulted in a marked decrease in J_{net}^{Na} (1.8±0.5 vs. 14.5±1.3 μeq/h·cm^2 in Ringer's solution, $P < 0.001$) secondary to a fall in J_{ms}^{Na} (11.0±0.8 vs. 25.6±1.2 μeq/h·cm^2 in Ringer's solution, $P < 0.001$). I_{sc} and conductance were also reduced. In the chloride-free Ringer solution sodium transport was similar in the control (1.1±0.4 μeq/h·cm^2) and sodium-depleted (1.8±0.5 μeq/h·cm^2) animals (Table I). Similarly, the absence of sodium in the sodium-depleted animals resulted in an inhibition of net chloride absorption (−0.1±0.3 vs. 7.2±1.8 μeq/h·cm^2, $P < 0.01$) and chloride transport in Na-free Ringer's solution was identical in control (−0.1±0.5 μeq/h·cm^2) and sodium-depleted (−0.1±0.3 μeq/h·cm^2) animals. These results are consistent with sodium depletion stimulation of neutral sodium-chloride absorption.

This augmentation of neutral sodium-chloride absorption could represent either dual ion exchanges (sodium–hydrogen and chloride–bicarbonate) or sodium-chloride cotransport. To distinguish between these two possibilities, experiments were performed in the sodium-depleted group with amiloride and bumetanide, inhibitors that affect either sodium-hydrogen exchange or sodium-chloride cotransport, respectively (16, 18, 19). Table II reveals that 1 mM mucosal amiloride produced only a minimal decrease in I_{sc} (−0.3±0.1 μeq/h·cm^2) in the sodium-depleted group but markedly reduced net sodium absorption by −6.0±1.1 μeq/h·cm^2 primarily due to a decrease in J_{ms}^{Na}. Amiloride also diminished net chloride absorption by −4.0±1.6 μeq/h·cm^2. In contrast, 0.1 mM mucosal bumetanide had no effect on either net sodium or net chloride transport (Table IV). These results suggest that the stimulation of neutral sodium-chloride absorption in the proximal colon of sodium-depleted rats is caused by an augmentation of dual ion exchanges (i.e., sodium–hydrogen and chloride–bicarbonate).

To assess whether sodium depletion is also stimulated electrogenic sodium transport (i.e., sodium transport that is inhibited by "low"-dose amiloride), the effect of 0.1 mM amiloride on sodium transport was also determined. These experiments were performed in chloride-free Ringer's solution in order to evaluate chloride-independent sodium transport. Both J_{net}^{Na} (2.3±0.9 vs. 2.0±0.5 μeq/h·cm^2) and I_{sc} (0.6±0.3 vs. 1.0±0.3 μeq/h·cm^2) were identical before and after the addition of 0.1 mM amiloride to the mucosal bathing solution. Thus, in marked contrast to the stimulation of electrogenic sodium transport in the distal colon in sodium depletion (5), amiloride did not inhibit sodium absorption in the proximal colon by blocking electrogenic sodium transport.

The effect of acetazolamide and DIDS on ion transport in the sodium depleted animals was also determined (Table III). The addition of 0.1 mM acetazolamide to both the mucosal and serosal bathing solutions resulted in significant decreases in both J_{ms}^{Na} (17.5±1.2 vs. 23.5±2.6 μeq/h·cm^2) and J_{net}^{Na} (8.6±1.3 vs. 13.9±2.4 μeq/h·cm^2) without altering J_{sm}^{Na}. Although J_{net}^{Cl} was reduced after the addition of acetazolamide, the change was not significant. The addition of 2.0 mM DIDS to the mucosal bathing solution did not alter J_{net}^{Na}, J_{net}^{Cl}, or I_{sc} (data not shown).

Discussion

The present experiments demonstrate that sodium and chloride transport in the proximal colon of the rat differs both qualitatively and quantitatively from that described in the distal segment by this laboratory. The rate of sodium absorption in the proximal segment is considerably greater than chloride absorption which is negligible (Table I). In the proximal segment sodium depletion does not induce amiloride-sensitive electrogenic sodium absorption as it does in the distal colon, but rather stimulates neutral sodium-chloride absorption. Comparison of electrolyte transport in the proximal and distal segments indicates that the proximal colon is a distinct epithelium with unique characteristics.

Several experimental observations support the conclusion that the mechanism of neutral sodium absorption in the control group is best explained by the presence of sodium–hydrogen exchange. (*a*) The demonstration that J_{net}^{Na} is substantially greater than I_{sc} provides evidence of neutral sodium absorption (Table I). (*b*) Because net chloride absorption is 0 and J_{net}^{Na} and J^R are approximately equal, neutral sodium absorption could be explained by sodium–hydrogen exchange. (*c*) The negative J^R could represent either cation secretion or anion absorption. Because J^R (−5.2±0.5 μeq/h·cm^2) is much greater than the rate of potassium secretion (0.20±0.02 μeq/h·cm^2) present in the proximal colon (9), proton secretion is the best explanation for J^R. (*d*) 1 mM amiloride, a concentration that in other epithelia

inhibits sodium–hydrogen exchange (16), reduced J^{Na}_{net} and J^R equally without affecting I_{sc} (Table II). (*e*) J^R is sodium-dependent in that J^R was abolished by the removal of sodium (Table I).

The removal of bicarbonate produced changes in ion transport similar to those induced by the addition of 1.0 mM amiloride because both the neutral component of net sodium transport ($J^{Na}_{net} - I_{sc}$) and J^R were not present in the bicarbonate-free Ringer's solution. As a result, I_{sc} (1.8 ± 0.2 μeq/h · cm^2 was totally accounted by J^{Na}_{net} (1.5 ± 0.4 μeq/h · cm^2) in the absence of bicarbonate. In that the bicarbonate-free Ringer's solution is oxygenated with 100% O_2, it cannot be determined whether these changes are due to the absence of bicarbonate, or CO_2, or both. It should be noted, however, that in the turtle bladder CO_2 induces electrogenic proton secretion (20). Whether CO_2 also stimulates proton secretion in the rat proximal colon albeit by another mechanism will require additional experiments.

Sodium–hydrogen exchanges have been described recently in several epithelia including the small intestine, the proximal tubule, and gallbladder; in some of these tissues sodium–hydrogen exchange may represent the primary mechanism of sodium absorption (18, 21–25). Turnberg et al. (21) concluded that bicarbonate-stimulated sodium absorption in the jejunum of normal subjects represented a sodium–hydrogen exchange process, and Sellin and DeSoignie (8) have reported that epinephrine stimulates sodium–hydrogen exchange in the rabbit proximal colon. Studies with apical membrane vesicles prepared from rat and rabbit ileum (22, 25) and rabbit proximal tubule (22) confirm the presence of the process which is electroneutral with a coupling ratio of 1.0 and is competitively inhibited by the diuretic amiloride. Direct demonstration of sodium–hydrogen exchange in colonic epithelium has not been reported.

The demonstration that the removal of chloride significantly reduced net sodium absorption in the control group was an unexpected finding. We have considered three possibilities to explain this observation but none are satisfactory. The first possibility is that zero chloride absorption is the result of oppositely directed chloride transport processes of equal magnitude. If the chloride absorptive process was neutral sodium-chloride absorption, the removal of chloride should inhibit sodium absorption (Table I). However, the nature of chloride secretory process is uncertain in that most chloride transport processes are either electrogenic and sodium-dependent (e.g., cyclic AMP-stimulated chloride secretion in the rabbit distal colon [26]) or electroneutral and sodium-independent (e.g., chloride–bicarbonate exchange in the rat and rabbit distal colon [4, 11]). Our results[2] are not consistent with either an electrogenic and sodium-dependent or an electroneutral and sodium-independent chloride secretory process but rather with an electroneutral, sodium-dependent process which has been described in the guinea pig ileum (27). However, if the chloride secretory process was sodium-dependent and of equal magnitude to the chloride-dependent sodium absorptive process, the removal of chloride should have had little effect on J^{Na}_{net}.

A second possibility, which was suggested by recent observations in the rabbit proximal colon (28), was also considered to explain the chloride dependence of sodium absorption despite the presence of 0 net chloride movement in the control group. In these studies (28) sodium absorption was chloride-dependent and chloride absorption was sodium-dependent, but the rate of net sodium and chloride absorption was higher at lower rather than at higher sodium and chloride concentrations, suggesting a type of "down" regulation of neutral sodium-chloride transport. A similar phenomena present in the rat proximal colon would explain our findings of chloride dependence of sodium absorption despite zero net chloride absorption. However, in the rat proximal colon J^{Na}_{net} was also 0 at 25 mM sodium-chloride (Budinger, M. E., and H. J. Binder, unpublished observations) indicating that this type of "down" regulation is not present in the proximal segment of rat colon.

It should be noted that the removal of either bicarbonate or chloride produces similar changes (i.e., inhibit both J^{Na}_{net} and J^R). Therefore, it is possible that a common mechanism may exist to account for the observations in both chloride-free and bicarbonate-free Ringer solution. For example, the removal of either chloride or bicarbonate may have resulted in intracellular alkalosis which would eliminate the driving force for sodium uptake. The failure, however, of mucosal SITS to alter ion transport is, however, surprising. Thus, there is not a totally satisfactory explanation to account for the chloride dependence of sodium absorption in the control group.

To study the effects of chronic hyperaldosteronism in experimental animals, it is not possible to infuse aldosterone into euvolemic animals with intact renal function in that marked potassium depletion will be induced, as well as the sodium-escape phenomena, in which epithelia exhibit a tolerance to the action of aldosterone to increase sodium absorption. Previous workers have shown that epithelial changes in sodium depleted animals with secondary hyperaldosteronism are caused primarily by the action of aldosterone. Will and associates (29) have shown that diverse chronic experimental conditions, including oral potassium loading, furosemide treatment, and sodium deprivation, in which hyperaldosteronism is induced, cause increased amiloride sensitivity in rat distal colon. Their experiments also demonstrated that the transport changes in rat distal colon of sodium deprived animals were completely inhibited by the aldosterone antagonist spironolactone, as were the changes induced by chronic aldosterone and desoxycorticosterone treatment (29). Previous studies have shown that chronic sodium deprivation does not cause an increase in the production rate of corticosterone (30). Taken together these data suggest that the epithelial changes observed in aldosterone-sensitive epithelia after prolonged sodium deprivation are caused primarily by the action of aldosterone although possible contribution by angiotensin II, renin, and unknown variables cannot be totally excluded.

The action of aldosterone has been studied in both amphibian tissue (31) and mammalian epithelia including the distal colon in rat and rabbit (5, 32–34). Despite extensive research, the cellular events responsible for the increase in net sodium absorption, net potassium secretion, and potential difference induced by aldosterone are controversial. In all tissues that have been previously examined, aldosterone stimulates amiloride-inhibitable electrogenic sodium absorption which is associated with increases in both apical sodium conductance and Na-K-ATPase activity (31). Although we are not aware of studies in other epithelia that reveal that sodium depletion or aldosterone stimulate neutral

2. The presence of an electrogenic, sodium-dependent chloride process would be associated with a marked decrease in I_{sc} after the removal of either chloride or sodium. The presence of an electroneutral, sodium-independent chloride secretory process would be associated with either unmasking of chloride secretion in sodium-free Ringer's solution or induction of chloride absorption by mucosal or serosal SITS. None of these possibilities was observed.

Table V. Effect of 0.1 mM Bumetanide on Unidirectional and Net Sodium and Chloride Fluxes of Sodium-depleted Animals

	Na flux			Cl flux						
	J_{ms}	J_{sm}	J_{net}	J_{ms}	J_{sm}	J_{net}	I_{sc}	PD	G	J^R
	$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$			$\mu eq/h \cdot cm^2$	mV	mS/cm^2	$\mu eq/h \cdot cm^2$
Period I	20.1	10.2	9.9	16.9	13.9	3.0	2.1	2.7	21.5	−4.8
	±1.7	±0.5	±1.8	±2.5	±1.4	±2.1	±0.3	±0.4	±1.5	±1.1
Period II	20.4	10.5	9.9	17.6	14.2	3.3	1.8	2.2	22.4	−4.8
	±1.7	±0.5	±1.9	±2.4	±1.4	±1.9	±0.2	±0.3	±1.7	±1.1
P*	NS	NS	NS	NS	NS	NS	<0.005	<0.005	<0.05	NS

Period I is the 15-min flux period before the addition of bumetanide to the mucosal solution. 12 min after the addition of bumetanide, a second 15-minute flux period was performed. See legend to Table I for additional details of experiment and definitions of abbreviations. Eight tissue pairs were studied. * Compared to period I.

sodium absorption, Al-Awqati et al. (35) found that aldosterone-induced proton secretion that was independent of sodium in the turtle urinary baldder and Stone et al. (36) recently reported that mineralocorticoids stimulate sodium-independent acid secretion in the rabbit medullary collecting duct.

The present in vitro experiments confirm our recent in vivo findings (10) that the effect of sodium depletion on sodium transport in the proximal colon is qualitatively distinct from its action on rat distal colon and extend these observations by providing insights into the mechanism of action of aldosterone on sodium and chloride absorption in the proximal colon. In contrast to the distal colon I_{sc} was only minimally elevated in the sodium-depleted group compared to controls (Table I), and amiloride produced only a small decrease in I_{sc} in the experimental group (Table II). These present studies indicate that the primary action of aldosterone on electrolyte transport in the proximal colon is stimulation of neutral sodium-chloride absorption because in the sodium-depleted animals sodium absorption was chloride-dependent and chloride absorption was sodium-dependent. Thus, sodium depletion resulted in an increase in sodium absorption in an epithelium without amiloride-sensitive sodium channels. To our knowledge, this is the first observation that sodium depletion (or aldosterone) stimulates sodium absorption by augmenting electroneutral sodium absorption and not inducing amiloride-sensitive, electrogenic sodium-transport.

Neutral sodium-chloride absorption is present in several epithelia and may represent either sodium-potassium-chloride cotransport or dual ion exchanges (37–40). In the present experiments, bumetanide did not inhibit sodium-chloride absorption in the sodium-depleted group (Table V) whereas 1 mM amiloride markedly reduced both J^{Na}_{net} and J^{Cl}_{net} (Table II). These results are most consistent with a model in which sodium depletion increases sodium–hydrogen and chloride–bicarbonate exchanges.[3] In that this conclusion is based primarily on differential inhibition by pharmacologic agents, demonstration of the presence of these ion exchange processes by more specific methods (e.g., apical membrane vesicles) will be required for confirmation.

Sellin and DeSoignie (8) recently described properties of ion transport in the rabbit proximal colon that were distinct from those in the rabbit distal colon. Both net sodium and chloride transport were 0 in the rabbit and were not affected by the removal of chloride or sodium respectively. Epinephrine produced comparable increases in both J^{Na}_{net} and J^{Cl}_{net}. This induction of sodium and chloride transport by epinephrine was partially prevented by the removal of chloride and by the addition of 1 mM amiloride and did not occur in the absence of sodium. The conclusion of these experiments was that epinephrine stimulated Na–H exchange in the proximal colon of the rabbit. Thus, both similarities and differences exist in the characteristics of sodium and chloride transport in the rabbit proximal colon compared to those in the proximal colon of the rat described in this present report.

Differences in ion transport processes in the rat colon have been reported (6, 9, 10, 13, 42), and significant differences in function appear to exist in various colonic segments of several other species including humans (1, 2, 7, 8, 43, 44). Thus, there may be more differences than similarities in the function of various segments of the large intestine. Failure to study only limited areas of the colon with homogeneous function may obscure important aspects of colonic ion transport.

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