Effects of Acid-Base Variables on Ion Transport in Rat Colon

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Abstract

Alterations in arterial acid-base variables have important effects on colonic electrolyte transport in vivo. To confirm the relative effects of these variables and to characterize the transport processes involved, we measured unidirectional ^{22}Na and ^{36}Cl fluxes across short-circuited, distal colonic mucosa of Sprague-Dawley rats. Stripped tissues were studied in Hepes buffer and in Ringer's solutions at HCO_3 concentrations of 11, 21, and 39 mM, and CO_2 tensions between 0 and 69.6 mmHg. Increases in P_{CO_2}, but not in either pH or HCO_3 concentration, caused similar increases in J^{Na}_{net} and J^{Cl}_{net} (net flux of sodium and chloride, respectively) from -0.2 ± 0.3 and -1.5 ± 0.4 μeq/cm^2 per h at $P_{CO_2} = 0$ to 6.8 ± 0.6 and 7.6 ± 0.7 μeq/cm^2 per h, respectively, at $P_{CO_2} = 69.6$ mmHg. These increases were accounted for by changes in *J*ms and were accompanied by small decreases in *I*sc. 1 mM acetazolamide decreased both J^{Na}_{net} and J^{Cl}_{net} and their responses to increases in CO_2. 0.75 mM luminal amiloride prevented the increase in sodium absorption, but did not affect the CO_2-induced increase in chloride absorption. In the presence of amiloride, CO_2 increased J^R (residual flux). 0.1 mM luminal furosemide did not affect the CO_2-induced increases in J^{Na}_{net} in the absence or presence of amiloride. Changes in HCO_3 concentration did not alter J^R. We conclude that ambient CO_2 effects active, electroneutral sodium absorption in the rat distal colon. The process stimulated by CO_2 is dependent on mucosal carbonic anhydrase activity and most likely represents Na/H and Cl/HCO_3 ion exchange.

Introduction

The in vivo observation that arterial acid-base variables have a major effect on intestinal electrolyte and water transport suggested a mechanism other than neurohumoral by which the body maintains electrolyte homeostasis (1–3). The distal colon of the rat is of particular interest in this regard, because its electrolyte transport systems are well characterized (4–9), and its response to arterial P_{CO_2} and HCO_3 concentration is specific and spans the physiological range of values for these variables. As in other transporting epithelia, the effects of CO_2 apparently were mediated through changes in the intracellular pH (pHi)[1] of the colonic epithelium (10, 11), and were dependent on mucosal carbonic anhydrase activity (12). The data also suggested that the transport processes affected were electroneutral and included both Na/H ion exchange and Cl/HCO_3 ion exchange (3, 11, 13).

Several questions were raised by these findings that could not be addressed in studies of intact animals. These included determining whether (*a*) the action of P_{CO_2} was entirely a local effect on the colonic epithelium, (*b*) the electroneutral sodium absorptive process stimulated by CO_2 reflected coupled NaCl cotransport as well as Na/H exchange, (*c*) the effect of the plasma HCO_3 concentration on net HCO_3 secretion reflected transcellular (as opposed to intercellular) HCO_3 movement, and (*d*) whether the responsive transport processes were pH-sensitive in the absence of ambient CO_2.

These issues were addressed in the present study by examining distal colonic tissue in the Ussing (E. W. Wright, Guilford, CT) ion flux chamber. This experimental approach was especially suitable because the response of this tissue to CO_2 is immediate, of significant magnitude, and completely reversible (2, 3, 11–13). As compared with in vivo studies, this method allows for the precise control and manipulation of acid-base variables in the absence of secondary cardiovascular and systemic neurohumoral influences. In addition, we could examine the role of various electrolyte transport processes by comparing the effects of P_{CO_2} in the presence and absence of specific inhibitors of each process.

Methods

Male Sprague-Dawley rats weighing 250–350 g were maintained on a standard diet with free access to water. While the rats were under pentobarbital sodium anesthesia (5 mg/100 g body wt), the distal 10 cm of descending colon was removed, rinsed with 0.9% saline, and placed over a glass rod. The tissue was stripped of the serosa and segments were mounted in modified Ussing half-chambers (E. W. Wright) exposing 1.12 cm^2 surface area. One tissue pair was obtained from each animal.

The transepithelial potential difference (PD) was measured using calomel electrodes connected to the half-chambers by bridges containing 3% agar constituted in the same Ringer's solution used as bathing solution. The PD was expressed as serosal side positive with respect to the mucosa. Tissues were studied under short-circuited conditions except for 1-s intervals every 100 s, during which bipolar pulses of 0.5 mV yielded electrical current values that were used to calculate tissue conductance (G). The short-circuit current (*I*sc) was passed across the tissue through 3% agar bridges via a voltage clamp (University of Iowa, Iowa City, IA). *I*sc, by convention, was given the same polarity as PD. Tissues were paired for ion flux studies on the basis of differences in G no greater than 25%.

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1. *Abbreviations used in this paper:* DIDS, 4,4′-diisothiocyanostilbene-2,2′ disulfonate; G, conductance; *I*sc, short-circuit current; J^{Cl}_{ms}, mucosal to serosal chloride flux; J^{Cl}_{net}, net chloride flux; *J*ms, mucosal to serosal flux; J^{Na}_{ms}, mucosal to serosal sodium flux; J^{Na}_{net}, net sodium flux; *J*net, net flux; *J*sm, serosal to mucosal flux; PD, potential difference; pHi, intracellular pH.

Unidirectional fluxes of Na and Cl were measured by adding 2 μCi of ^{22}Na and 1 μCi of ^{36}Cl (100 Ci/g sp act; New England Nuclear, Boston, MA) to the mucosal side of one member of each tissue pair and the serosal side of the other. After an initial 15–30-min equilibration period, samples of the hot side were collected at the beginning and end of a 45-min experimental period for determination of each isotope's specific activity. Samples of the cold side were taken every 15 min during the 45-min period for determination of unidirectional ion flux. Samples from both sides were replaced with the appropriate Ringer's solutions. Ready-Solv HP (Beckman Instruments, Inc., Fullerton, CA) was added to each sample and counts were obtained in a liquid scintillation system (LS-3133P) and a gamma counter (both obtained from Beckman Instruments, Inc.). Mucosal to serosal (*J*ms) and serosal to mucosal (*J*sm) fluxes were calculated by standard formulae (4), and net flux (*J*net) was calculated as the difference between them (*J*ms − *J*sm). The residual flux (J^R) was calculated by $J^R = Isc - (J^{Na}_{net} - J^{Cl}_{net})$.

Acid-base variables

Paired tissues were bathed in 10 ml of identical Ringer's solutions at 37°C containing (in millimoles per liter): NaCl, 96; $NaHCO_3$, 21; KCl, 4; Na_2HPO_4, 2.4; NaH_2PO_4, 0.4; Na gluconate, 18; $CaSO_4$, 1.0; $MgSO_4$, 1.2; and glucose, 10. Tissue pairs were studied during two or four consecutive 45-min experimental periods in which bathing solution HCO_3 concentration and pH, P_{CO_2} and pH, or HCO_3 concentration and P_{CO_2} were altered. Bicarbonate concentrations were altered by substituting Na gluconate or Hepes buffer for $NaHCO_3$ over the range 0 to 39 mM. In HCO_3-free Ringer's solution, Hepes buffer was titrated with 1 M HCl or NaOH, and the solution was gassed with 100% O_2. In HCO_3-H_2CO_3-buffered solutions, tissues were gassed with either room air (P_{CO_2} = 0 mmHg), 3% CO_2/97% O_2 (P_{CO_2} = 21.9 mmHg), 5% CO_2/95% O_2 (P_{CO_2} = 34.5 mmHg), or 11% CO_2/89% O_2 (P_{CO_2} = 69.6 mmHg). In preliminary time-control experiments, stable levels of sodium and chloride flux were observed for more than 6 h, and changes in transport induced by the acid-base variables were fully reversible.

The pH and P_{CO_2} of the bathing solutions studied in consecutive periods were measured before isotope addition with a Radiometer BMS 3 MK 2 system; with a (PHM 73 acid-base analyzer; London, Cleveland, OH). Bicarbonate concentration was calculated by the Henderson-Hasselbach equation as previously described (1, 2). In salt solutions, $pK' = 6.33 - 0.52\,(I)^{1/2} \pm 0.6\,(I)$, where 6.33 is the *p*K of the H_2CO_3-HCO_3 buffer system in water, and *I* is the sodium plus potassium concentrations in moles per liter (2, 14). To correct *p*K′ for pH, an empiric factor was determined in this laboratory: $pK'' = pK' - \log(1 + \log^{pH-8.7})$. The CO_2 solubility in salt solutions was adjusted for ionic strength: alpha = $10^{-1.482-0.085(I)}$ (2, 14).

Inhibitors

We then examined whether inhibitors affected the change in transport caused by the acid-base variables. Electrolyte transport was measured at a bathing solution HCO_3 concentration of 21 mM during consecutive periods of P_{CO_2} = 21.6 and 69.6 mmHg before and after the addition of the specific inhibitor.

Acetazolamide. Sodium acetazolamide (Lederle Parenterals, Inc., Carolina, PR) was added to both mucosal and serosal bathing solutions at a final concentration of 0.1 or 1.0 mM. Electrolyte transport was measured at a bathing solution HCO_3 concentration of 21 mM during consecutive periods of P_{CO_2} = 21.6 and 58.6 mmHg, and P_{CO_2} = 21.6 and 69.6 mmHg.

Amiloride. Amiloride (Merck Sharp & Dohme Research Laboratories, West Point, PA) was added to the mucosal bathing solution at a final concentration of 0.75 mM. The effect of amiloride also was studied in a bathing solution in which choline chloride was substituted for sodium chloride so as to reduce the sodium concentration to 50 mM.

Furosemide. Furosemide (Sigma Chemical Co., St. Louis, MO) was added to the mucosal bathing solution at a final concentration of 0.1 mM.

Amiloride and furosemide. To determine whether amiloride and furosemide were additive in their effects and whether the effect of furosemide was affected by the presence of amiloride, 0.75 mM amiloride and 0.1 mM furosemide were both added to the mucosal bathing solution. The effects of increasing P_{CO_2} were then compared with the changes caused by CO_2 in the presence of amiloride or furosemide.

4,4′-Diisothiocyanostilbene-2,2′ disulfonate (DIDS). DIDS was added to the mucosal side of a 21-mM HCO_3 Ringer's solution at a final concentration of 0.1 mM or 1 mM.

Statistics

Calculated values for the 3 15-min samples for each condition were averaged and the values for each experimental group were expressed as the mean±SE. Statistical analyses consisted of paired and unpaired, two-tailed *t* tests, one-way analysis of variance, and linear regression analysis by least squares (15). Correlation coefficients were calculated from the mean values of the experimental groups. Corrections were made for multiple *t* tests when appropriate, and a $2P$ value < 0.05 was considered significant.

Results

Acid-base variables

The effects of varying the P_{CO_2} on electrolyte transport at a bathing solution HCO_3 concentration of 21 mM are shown in Table I. At P_{CO_2} = 0 mmHg, net Na absorption was zero and net chloride secretion was observed. The residual ion flux, J^R, also was zero. Net Na and Cl absorption were noted at P_{CO_2} = 21.6 mmHg, and progressively increased so that at P_{CO_2} = 69.6 mmHg, net Na and Cl absorption had increased ~ 60%. The increase in absorption was accounted for by an increase in *J*ms rather than by a reduction in *J*sm. Increases in P_{CO_2} between 0 and 69.6 mmHg did not alter PD, but did increase J^R ($P < 0.05$) and decrease the *I*sc ($P < 0.01$) and conductance ($P < 0.02$). These changes in ion flux and G were completely reversible, and occurred regardless of the order in which P_{CO_2} was altered.

To determine to what extent the observed responses were effects of P_{CO_2} and not pH, we measured electrolyte fluxes at bathing solution HCO_3 concentrations of 39 and 11 mM at a constant P_{CO_2} of 34.5 mmHg. As shown in Table II, there were no significant differences in the net absorption of sodium or chloride in these solutions of markedly different HCO_3 concentration and pH. Indeed, the pH range examined in this experiment (7.65–7.14) was similar to that caused by changing P_{CO_2} from 21.6 to 69.6 mmHg (compare Tables I and II). There also was no effect of pH or HCO_3 concentration on J^R, although J^R tended to be greater in 39 mM HCO_3 than in 11 mM HCO_3 Ringer's solution.

Nevertheless, because these data were obtained (by necessity) in separate groups of tissues, a small effect of pH may have been obscured by intertissue variability. We therefore measured ion flux in a HCO_3-free Hepes-Ringer's solution gassed with 100% O_2 that was titrated during consecutive periods with HCl or NaOH. As shown in Table II, J^{Na}_{ms}, J^{Na}_{net}, J^{Cl}_{ms}, and J^{Cl}_{net} were markedly reduced in the virtual absence of ambient HCO_3 and CO_2. J^R was zero under these conditions. When bathing solution pH was reduced from 7.59 to 7.09, very small but significant changes were noted in *I*sc, PD, G, and the mucosal to serosal and net fluxes of Na and Cl. These changes were qualitatively similar to, although much smaller than, those seen when a similar pH change was induced by altering P_{CO_2} (Table I). In addition, the decreases in *I*sc and

Table I. Effect of CO_2 on Colonic Electrolyte Transport

	P_{CO_2}	pH	Isc	PD	G	Na flux Jms	Na flux Jsm	Na flux Jnet	Cl flux Jms	Cl flux Jsm	Cl flux Jnet	J^R
	mmHg		*μeq/cm² per h*	*mV*	*mS/cm²*	*μeq/cm² per h*			*μeq/cm² per h*			
	0	8.42 ±0.03	1.8 ±0.1	4.4 ±0.1	11.1 ±0.4	5.9 ±0.3	6.1 ±0.3	−0.2 ±0.3	7.4 ±0.4	8.9 ±0.6	−1.5 ±0.4	0.4 ±0.5
	21.6 ±0.7	7.63 ±0.01	1.6 ±0.2	5.0 ±0.5	8.9 ±0.6	9.2 ±0.5	5.0 ±0.3	4.2 ±0.4	12.8 ±0.5	8.0 ±0.3	4.8 ±0.5	2.2 ±0.3
	34.5 ±0.8	7.41 ±0.01	1.4 ±0.2	4.7 ±0.7	8.5 ±0.6	10.1 ±0.8	4.7 ±0.2	5.5 ±0.8	14.6 ±0.8	8.0 ±0.4	6.6 ±0.8	2.5 ±0.4
	69.6 ±1.2	7.10 ±0.01	0.9 ±0.1	3.2 ±0.5	7.9 ±0.5	11.7 ±0.9	4.8 ±0.3	6.8 ±0.6	16.5 ±0.7	9.0 ±0.4	7.6 ±0.7	1.7 ±0.3
P*	<0.001	<0.001	<0.025			<0.01		<0.001	<0.001		<0.001	<0.05

Values are means±SE. Number of tissue pairs studied at a P_{CO_2} of 0 = 4, at a P_{CO_2} of 21.6 = 17, at a P_{CO_2} of 34.5 = 15, and at a P_{CO_2} 69.6 = 17. Data were obtained in separate groups of tissue pairs bathed in 140 mM Na and 21 mM HCO_3-Ringer's solutions at one or more CO_2 tensions. * Four experimental groups were compared by one-way analysis of variance.

PD in Hepes buffer were entirely accounted for by increases in net Cl transport since as shown in Table II, $Isc = J^{Na}_{net} - J^{Cl}_{net}$ and $J^R = 0$.

We then examined the possibility that the concentration of HCO_3 altered the effect of CO_2 on Na and Cl absorption. We measured ion fluxes in the 39-mM HCO_3 Ringer's solution at CO_2 = 69.6 mmHg (pH 7.35) and in the 11-mM HCO_3 Ringer's solution at P_{CO_2} = 21.6 mmHg (pH 7.35). The differences in net Na flux (1.3 μeq/cm² per h), net Cl flux (3.6 μeq/cm² per h), Isc (−0.9 μeq/cm² per h), and G (0.1 mS/cm²) at these CO_2 tensions were similar to the differences observed between similar P_{CO_2} levels in the 21-mM HCO_3 Ringer's solution shown in Table I.

The relative effects of bathing solution P_{CO_2}, pH, and HCO_3 concentration on ion flux were confirmed by correlating these variables in all 75 tissue pairs in all the acid-base groups described above (except the groups exposed to room air or bathed in Hepes-Ringer's solution). As shown in Fig. 1, net sodium absorption correlated only with bathing solution P_{CO_2} ($r = 0.83$, $P < 0.02$) and did not correlate with either pH or HCO_3 concentration. When unidirectional fluxes were examined, J^{Na}_{ms} correlated with P_{CO_2} ($r = 0.97$, $P < 0.001$). The slopes of both of these relations were 0.04, indicating that for each 10-mmHg increment in P_{CO_2}, J^{Na}_{net} flux increased 0.4 μeq/cm² per h.

Although the relation between CO_2 and chloride absorp-

Table II. Effect of pH on Colonic Electrolyte Transport in HCO_3 and Hepes-buffered Ringer's Solutions

	HCO_3	P_{CO_2}	pH	Isc	PD	G	Na flux Jms	Na flux Jsm	Na flux Jnet	Cl flux Jms	Cl flux Jsm	Cl flux Jnet	J^R
	mM	*mmHg*		*μeq/cm² per h*	*mV*	*mS/cm²*	*μeq/cm² per h*			*μeq/cm² per h*			
HCO_3 Ringer's	39	35.8 ±1.0	7.65 ±0.01	1.3 ±0.1	3.8 ±0.4	9.2 ±0.6	10.2 ±0.7	5.7 ±0.7	4.5 ±0.8	13.2 ±0.7	7.4 ±0.7	5.8 ±0.7	2.6 ±0.4
	11	34.3 ±0.7	7.14 ±0.01	1.4 ±0.2	5.2 ±0.8	7.8 ±2.1	10.4 ±0.7	5.4 ±0.3	5.1 ±0.8	15.8 ±0.9	10.4 ±0.4	5.4 ±0.8	1.7 ±0.4
P*			<0.001		<0.001					<0.05	<0.001		
Hepes Ringer's	0	0	7.59 ±0.01	1.7 ±0.1	6.5 ±0.6	7.0 ±0.2	5.5 ±0.2	4.4 ±0.2	1.1 ±0.4	8.1 ±0.7	8.4 ±0.5	−0.2 ±0.8	0.3 ±0.5
	0	0	7.09 ±0.01	1.3 ±0.2	5.7 ±0.7	6.2 ±0.3	6.1 ±0.4	4.0 ±0.3	2.2 ±0.6	8.9 ±0.7	7.7 ±0.5	1.2 ±0.8	0.3 ±0.3
P‡			<0.001	<0.005	<0.05	<0.001	<0.05	<0.01	<0.01	<0.025	<0.005	<0.005	

Values are means±SE. Number of tissue pairs studied at 39 mM HCO_3-Ringer's solution = 12, 11 mM HCO_3-Ringer's = 14, and 21 mM Hepes = 9. Data for HCO_3-Ringer's solution were obtained in separate groups of tissue pairs bathed in 39 HCO_3-Ringer's solution or 11 mM HCO_3-Ringer's solution. Data for Hepes-Ringer's solution were obtained in one group of tissues bathed in 21 mM Hepes-Ringer's solution titrated during consecutive periods with HCl or NaOH. * Unpaired *t* test, ‡ paired *t* test.

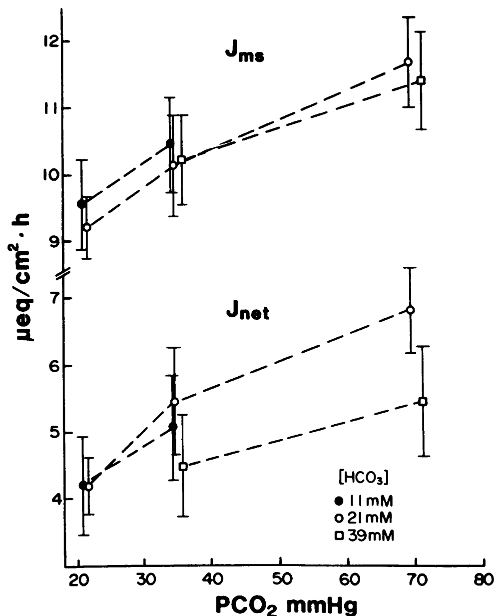

Figure 1. Relation of sodium absorption to bathing solution P_{CO_2}. J^{Na}_{ms} and J^{Na}_{net} were measured in Ringer's solutions containing 11, 21, or 39 mM HCO_3 during consecutive periods in which P_{CO_2} was altered as indicated by the dashed lines. Both J^{Na}_{ms} and J^{Na}_{net} increased with increasing P_{CO_2}. Values are means±SE.

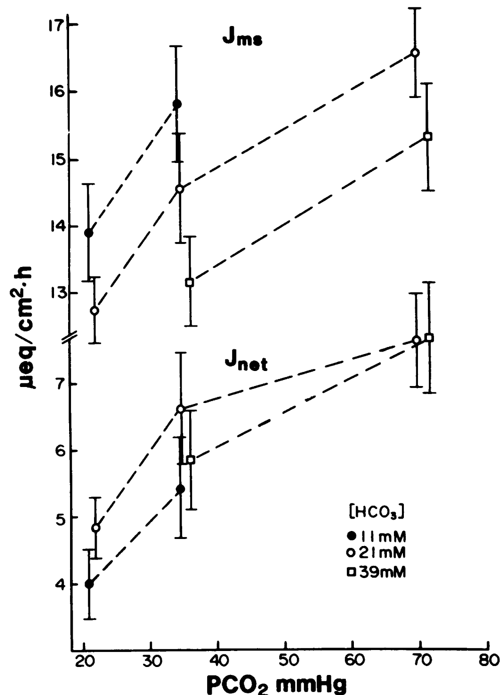

Figure 2. Relation of chloride absorption to bathing solution P_{CO_2}. J^{Cl}_{ms} and J^{Cl}_{net} were measured in Ringer's solutions containing 11, 21, or 39 mM HCO_3 during consecutive periods in which P_{CO_2} was altered as indicated by the dashed lines. Both J^{Cl}_{ms} and J^{Cl}_{net} increased with increasing P_{CO_2}. Values are means±SE.

tion appeared to be curvilinear (Fig. 2), when analyzed by linear regression analysis, similar results were obtained for chloride flux. Net chloride absorption correlated with P_{CO_2} ($r = 0.92$, $P < 0.005$), but not with either pH or HCO_3 concentration. A correlation also was found for J^{Cl}_{ms} and P_{CO_2} ($r = 0.72$, $P < 0.05$). The slopes of these relations were similar to the slope relating sodium flux to P_{CO_2}. There was an ~ 0.6 μeq/cm² per h increase in J^{Cl}_{net} per 10-mmHg increase in P_{CO_2}.

Inhibitors

In another series of experiments, inhibitors of various electrolyte transport processes were added to 21 mM HCO_3 Ringer's bathing solution and the effect of increasing CO_2 tension was measured. Alterations in Na and Cl fluxes in the presence and absence of the inhibitor were then compared.

Acetazolamide. To examine the effect of inhibiting mucosal carbonic anhydrase activity, 0.1 mM sodium acetazolamide was added to both mucosal and serosal bathing solutions. At P_{CO_2} = 21.6 mmHg, this addition reduced *J*ms and *J*net for chloride ~ 50% (Table III). As shown in Table IV, however, the increments in net sodium and chloride flux caused by increasing P_{CO_2} from 21.6 to 69.6 mmHg, although somewhat smaller, were not significantly different in the presence and absence of acetazolamide. The presence of acetazolamide nevertheless was associated with an increase in J^R in response to this increase in P_{CO_2}.

This partial effect of acetazolamide suggested the possibility that the 48-mmHg increase in CO_2 tension (69.6 minus 21.6 mmHg) had overcome the effects of 0.1 mM acetazolamide. The effect of increasing P_{CO_2} from 21.6 to 58.6 mmHg on net sodium and chloride fluxes was therefore compared with the increments caused by increasing P_{CO_2} from 21.6 to 69.6 mmHg. In fact, these increments were similar. When P_{CO_2} was raised from 21.6 to 58.6 mmHg in the presence of 0.1 mM acetazolamide, J^{Na}_{net} increased from 2.7±0.8 to 5.0±1.2 μeq/cm² per h and J^{Cl}_{net} increased from 2.8±0.7 to 6.7±1.0 μeq/cm² per h (compare with Tables III and IV). These findings suggested that a 0.1-mM concentration of acetazolamide was insufficient to completely inhibit mucosal carbonic anhydrase in our system. Consequently, we repeated the experiment in the presence of 1.0 mM acetazolamide. Under these conditions, *J*ms for both sodium and chloride were markedly reduced, resulting in zero net sodium absorption and net chloride secretion at P_{CO_2} = 21.6 mmHg. In addition, *I*sc tended to decrease and J^R changed direction (Table III). When P_{CO_2} was increased from 21.6 to 69.6 mmHg, the increases in *J*ms for both Na and Cl were significantly smaller than in the absence of acetazolamide (Table IV).

Amiloride. The participation of electrogenic Na transport and Na/H exchange in the colonic response to P_{CO_2} were examined by adding 0.75 mM amiloride to the mucosal bathing solution. At a CO_2 tension of 21.6 mmHg, amiloride decreased J^{Na}_{ms} 43%, J^{Na}_{net} 69%, and J^{Cl}_{ms} 23% (Table III). As shown in Table IV, the presence of amiloride markedly reduced but did not completely prevent the increments in J^{Na}_{ms} and J^{Na}_{net} produced by increasing the P_{CO_2} to 69.6 mmHg. In addition, the increments in J^{Cl}_{ms} and J^{Cl}_{net} caused by increasing P_{CO_2} were unaffected by the presence of amiloride. These increments in chloride flux were associated with increases in J^R that were not observed in the absence of amiloride.

We also studied amiloride in a HCO_3-Ringer's bathing solution in which the sodium concentration was reduced to 50 mM (Table V). In the absence of amiloride, increases in P_{CO_2}

Table III. Effect of Various Inhibitors on Colonic Electrolyte Transport at a P_{CO_2} of 21.6 mmHg

Addition	*Isc*[*]	PD	G[‡]	Na flux *Jms*[‡]	Na flux *Jsm*	Na flux *Jnet*[‡]	Cl flux *Jms*[‡]	Cl flux *Jsm*	Cl flux *Jnet*[‡]	J^R
	μeq/cm² per h	*mV*	*mS/cm²*	*μeq/cm² per h*			*μeq/cm² per h*			
None	1.6 ±0.2	5.0 ±0.5	8.9 ±0.6	9.2 ±0.5	5.0 ±0.3	4.2 ±0.4	12.8 ±0.5	8.0 ±0.3	4.8 ±0.4	2.2 ±0.3
Acetazolamide (0.1 mM)	1.3 ±0.1	4.8 ±0.7	8.4 ±0.7	7.7 ±0.7	5.1 ±0.5	2.6 ±0.6	10.2[§] ±0.7	8.1 ±0.4	2.1[‖] ±0.5	0.8 ±0.6
Acetazolamide (1.0 mM)	0.9 ±0.2	4.2 ±0.5	5.7[§] ±0.4	3.8[‖] ±0.1	3.8 ±0.2	0.0[‖] ±0.2	5.3[‖] ±0.4	7.6 ±0.7	−2.3[‖] ±0.5	−1.3[‖] ±0.5
Amiloride (0.75 mM)	1.2 ±0.1	6.2 ±0.4	5.4[‖] ±0.5	5.0[‖] ±0.5	3.7 ±0.4	1.3[§] ±0.7	10.0[§] ±0.7	6.9 ±0.7	3.1 ±0.8	3.1 ±0.6
DIDS (0.1 mM)	2.0 ±0.0	7.4 ±1.0	7.7 ±0.4	6.3[§] ±0.7	4.5 ±0.3	1.8[§] ±0.8	10.0[§] ±0.7	7.9 ±0.5	2.1 ±1.2	2.3 ±0.4
Furosemide (0.1 mM)	1.6 ±0.3	6.3 ±1.2	7.3 ±0.4	6.5[§] ±0.7	4.6 ±0.4	1.9[§] ±1.1	9.6[§] ±0.8	7.6 ±0.4	2.0[§] ±0.6	1.8 ±0.6
Amiloride (0.75 mM) Furosemide (0.1 mM)	1.7 ±0.3	6.4 ±0.9	7.1 ±0.3	4.1[‖] ±0.4	4.5 ±0.3	−0.4[‖] ±0.5	8.8[‖] ±0.5	9.4 ±0.5	−0.6[‖] ±0.6	1.6 ±0.6

Values are means±SE. Number of tissue pairs studied with no addition = 17, 0.1 mM acetazolamide = 15, 1.0 mM acetazolamide = 6, 0.75 mM amiloride = 8, 0.1 mM DIDS = 6, 0.1 mM furosemide = 6, and 0.75 mM amiloride plus 0.1 mM furosemide = 7. Separate groups of tissue pairs were studied in 140 mM Na and 21 mM HCO_3-Ringer's solution at a CO_2 tension of 21.6 mmHg (pH 7.63). * $P < 0.002$, ‡ $P < 0.001$ when the mean values for all groups were compared by one-way analysis of variance. § $P < 0.05$, ‖ $P < 0.005$ when compared with addition: none by unpaired *t* test.

Table IV. Effect of Increasing P_{CO_2} on Colonic Electrolyte Transport in the Presence of Various Inhibitors

Addition	Δ*Isc*	ΔPD	ΔG	ΔNa flux *Jms*	ΔNa flux *Jsm*	ΔNa flux *Jnet*	ΔCl flux *Jms*	ΔCl flux *Jsm*	ΔCl flux *Jnet*	ΔJ^R
	μeq/cm² per h	*mV*	*mS/cm²*	*μeq/cm² per h*			*μeq/cm² per h*			
None	−0.7 ±0.1	−1.8 ±0.4	−1.0 ±0.2	2.5 ±0.5	−0.2 ±0.1	2.6 ±0.5	3.8 ±0.4	1.0 ±0.3	2.8 ±0.5	−0.6 ±0.3
Acetazolamide (0.1 mM)	−0.6 ±0.2	−2.7 ±0.5	−1.1 ±0.2	1.5 ±0.3	−0.5 ±0.2	2.0 ±0.4	3.0 ±0.7	−0.6* ±0.4	3.6 ±0.7	1.0* ±0.6
Acetazolamide (1.0 mM)	−0.4 ±0.1	−1.5 ±0.4	−0.5 ±0.2	0.9* ±0.2	−0.4 ±0.3	1.3* ±0.4	1.1* ±0.3	−1.5* ±0.5	2.6 ±0.4	0.9* ±0.4
Amiloride (0.75 mM)	−0.7 ±0.9	−2.9 ±0.5	−0.8 ±0.7	0.6* ±0.2	−0.5 ±0.2	1.1* ±0.2	3.2 ±0.2	0.4 ±0.3	2.8 ±0.3	0.9* ±0.3
DIDS (0.1 mM)	−1.0 ±0.2	−3.3* ±0.9	−1.3 ±0.2	2.5 ±0.7	−0.1 ±0.3	2.6 ±0.9	3.9 ±0.8	0.2 ±0.6	3.6 ±0.5	0.0 ±0.5
Furosemide (0.1 mM)	−0.6 ±0.2	−1.8 ±0.6	−0.9 ±0.2	1.9 ±0.4	−0.5 ±0.2	2.4 ±0.4	2.9 ±0.6	0.2 ±0.3	2.8 ±0.5	−0.2 ±0.2
Amiloride (0.75 mM) Furosemide (0.1 mM)	−0.7 ±0.2	−2.3 ±0.7	−0.8 ±0.2	0.2* ±0.3	−0.5* ±0.1	0.7* ±0.3	2.5* ±0.5	0.5 ±0.3	2.0 ±0.5	0.5* ±0.4

Values are means±SE. Number of tissue pairs studied with no addition = 17, with 0.1 mM acetazolamide = 15, with 1.0 mM acetazolamide = 6, with 0.75 mM amiloride = 8, with 0.1 mM DIDS = 6, with 0.1 mM furosemide = 6, and with 0.75 mM amiloride plus 0.1 mM furosemide = 7. Separate groups of tissue pairs were studied in 140 mM Na and 21 mM HCO_3-Ringer's solution at CO_2 tensions of 21.6 (pH 7.63) and 69.6 mmHg (pH 7.10). Data (deltas) represent the differences between values at 69.6 mmHg minus values at 21.6 mmHg. Data for addition: none were derived from experiments presented in Table I. * $P < 0.05$ when compared with addition: none by unpaired *t* test.

caused qualitatively and quantitatively similar changes in ion flux, as were observed in a bathing solution containing 140 mM sodium (compare Tables IV and V). The effect of amiloride at a P_{CO_2} of 21.6 mmHg also was similar at 50 and 140 mM sodium: amiloride reduced J_{net}^{Na} 70% and J_{net}^{Cl} 47%, and increased J^R. Nevertheless, when the P_{CO_2} was raised in 50 mM sodium Ringer's solution, amiloride completely inhibited the increases in J_{ms}^{Na} and J_{net}^{Na}.

Furosemide. To determine whether a cotransport process was involved in the colonic response to CO_2, 0.1 mM furosemide was added to the mucosal bathing solution. At P_{CO_2} = 21.6 mmHg, furosemide decreased J_{net}^{Na} 55% and J_{net}^{Cl} 67%, respectively (Table III). This effect was additive to that of amiloride: the mucosal addition of both amiloride and furosemide at P_{CO_2} = 21.6 mmHg resulted in J_{net}^{Na} ($P < 0.05$) and J_{net}^{Cl} ($P < 0.005$) values that were significantly lower than for amiloride alone. In fact, J_{net}^{Na} and J_{net}^{Cl} did not differ from zero under these conditions.

Despite this effect of furosemide at P_{CO_2} = 21.6 mmHg, a furosemide-inhibitable process did not appear to participate in the colonic response to P_{CO_2}. As shown in Table IV, furosemide did not alter the increments in J_{net}^{Na} or J_{net}^{Cl} caused by increasing bathing solution P_{CO_2} to 69.6 mmHg. Nevertheless, it was possible that in the presence of amiloride, a role for this process might be revealed. The effect of CO_2, therefore, was measured in tissues exposed to mucosal furosemide in a 21-mM HCO_3 Ringer's solution containing amiloride. As shown in Table IV, the increments in J_{net}^{Na} and J_{net}^{Cl} in response to increasing P_{CO_2} under these conditions were no different than in tissues exposed to similar P_{CO_2} changes in the presence of amiloride alone: ΔJ_{net}^{Na} 0.7±0.3 vs. 1.1±0.2 μeq/cm^2 per h, NS; ΔJ_{net}^{Cl} 2.0±0.5 vs. 2.8±0.3 μeq/cm^2 per h, NS).

DIDS. The addition of DIDS to the mucosal bathing solution at a final concentration of 0.1 mM reduced J_{ms}^{Na}, J_{net}^{Na}, and J_{ms}^{Cl} at P_{CO_2} = 21.6 mmHg. A similar reduction in J_{net}^{Cl} did not reach statistical significance. Nevertheless, DIDS at 0.1 mM or at a 10-fold higher concentration did not alter the increments in J_{net}^{Na} or J_{net}^{Cl} induced by increasing the P_{CO_2} to 69.6 mmHg (data for 0.1 mM DIDS shown in Table IV).

Discussion

In previous studies we reported that physiologic changes in arterial P_{CO_2} have major effects on electrolyte and water absorption in the rat colon (1–3). The in vivo experimental conditions, however, allowed only tentative conclusions regarding the specificity and site of action of CO_2 (11, 13). The current findings confirm that the action of CO_2 on sodium and chloride absorption is predominant among the acid-base variables, and represents a local effect of CO_2 on the intestinal mucosa. A significant effect of pH was ruled out by the absence of a correlation between bathing solution pH and either J_{net}^{Na} or J_{net}^{Cl}, and by the minimal effect of pH on ion flux (between 7.09 and 7.65) when P_{CO_2} remained unchanged. The overall magnitude of the CO_2 effect was of interest as well: J_{net}^{Na} and J_{net}^{Cl} increased ~ 0.5 μeq/cm^2 per h per 10-mmHg increase in P_{CO_2}. These increments are equivalent to the 60% increases in sodium and chloride absorption observed in vivo over a similar range of CO_2 tensions (2 μeq/cm length per h per 10-mmHg change in arterial P_{CO_2}) (2, 3).

The colonic transport pathways affected by CO_2 appear to be active, electroneutral sodium and chloride absorptive processes. The importance of electroneutral absorption was originally suggested during in vivo studies of rat colon by the minimal changes in PD, and the equivalent increases in net sodium and chloride absorption in response to increases in arterial P_{CO_2} (2, 3, 12). In addition, the rabbit distal colon, which absorbs sodium by conductive, electrogenic pathways (16), did not respond at all to alterations in arterial P_{CO_2} (17). The nature of this electroneutral process remained uncertain, however, although the in vivo data were most consistent with the participation of Na/H and Cl/HCO_3 ion exchange processes (3, 13). It was presumably these exchangers, located along the luminal membranes of colonic epithelial cells, that responded to the changes in pHi and intracellular HCO_3 concentrations that mediated the CO_2 effect (11).

In the present study, we examined the role of the various transport pathways by comparing the increments in ion flux caused by CO_2 in the presence and absence of several transport

Table V. Effect of CO_2 and Amiloride on Colonic Electrolyte Transport in Low Sodium Ringer's Solution

Addition	P_{CO_2}	pH	*Isc*	PD	G	Na flux *Jms*	Na flux *Jsm*	Na flux *Jnet*	Cl flux *Jms*	Cl flux *Jsm*	Cl flux *Jnet*	J^R
	mM		*μeq/cm² per h*	*mV*	*mS/cm²*	*μeq/cm² per h*			*μeq/cm² per h*			
None	21.3	7.64	1.5	8.3	4.6	8.7	1.8	6.9	12.8	7.3	5.5	0.1
	±0.6	±0.01	±0.3	±1.1	±0.6	±2.3	±0.4	±2.1	±1.8	±0.7	±1.3	±1.4
	71.1	7.11	0.7	5.1	3.9	10.5	1.6	8.9	16.3	8.0	8.3	0.1
	±0.3	±0.0	±0.1	±0.8	±0.6	±2.5	±0.3	±2.3	±2.2	±0.5	±2.0	±1.8
*P**	<0.001	<0.001	<0.02	<0.001	<0.02	<0.005		<0.005	<0.001	<0.05	<0.01	
Amiloride (0.75 mM)	21.3	7.64	1.2	6.8	4.1‡	3.8‡	1.7	2.1§	10.2§	7.4	2.9§	1.9
	±0.6	±0.01	±0.3	±0.9	±0.6	±0.8	±0.4	±0.6	±0.9	±0.7	±0.5	±0.5
	71.1	7.11	0.5	3.4	3.7	3.9	1.4	2.4	12.9	8.4	4.5	2.8
	±0.3	±0.0	±0.1	±0.6	±0.4	±0.9	±0.3	±0.7	±1.4	±0.7	±1.0	±0.8
*P**	<0.001	<0.001		<0.01					<0.01	<0.05	<0.05	

Values are means±SE. Number of tissue pairs studied = 7. Tissue pairs were studied in 50 mM Na, 21 mM HCO_3 Ringer's solution at CO_2 tensions of 21.3 and 71.1 mmHg in the presence and absence of 0.75 mM luminal amiloride. * Paired *t* test. ‡ $P < 0.02$, § $P < 0.05$ when compared with addition: none at P_{CO_2} = 21.3 mmHg by paired *t* test.

inhibitors. This method allowed us to differentiate between the presence of a transport process and its participation in the colonic response to CO_2 (12). The most dramatic example of such a dissociation was our finding that 0.1 mM furosemide inhibited both sodium and chloride absorption ~ 55% at P_{CO_2} = 21.6 mmHg, but did not reduce the increments in absorption caused by increasing the P_{CO_2}. We interpret this finding to mean that an electroneutral sodium chloride absorptive process inhibited by furosemide, although present in rat colonic epithelial cells and an important pathway for sodium absorption, does not respond to alterations in P_{CO_2}. It is not clear at present whether furosemide inhibited a sodium chloride absorptive process distinct from Na/H and Cl/HCO_3 ion exchange, or whether these antiporters are sensitive to furosemide (but not CO_2) under certain experimental and physiologic conditions. Indeed, in one recent study using apparently similar methodology, furosemide-sensitive sodium chloride absorption was not found in the rat distal colon (18).

We may compare the effects of furosemide on colonic absorption with those of luminal amiloride. At a P_{CO_2} of 21.6 mmHg, amiloride also markedly inhibited sodium absorption. In fact, at low CO_2 tensions, the combination of amiloride and furosemide reduced net sodium and chloride absorption to zero. However, whereas CO_2 and furosemide apparently affect separate sodium absorptive pathways, CO_2 and amiloride modulate the same process. We found that the increment in net sodium absorption caused by an increase in P_{CO_2} was markedly diminished in the presence of amiloride. At luminal bathing solution concentrations reported to inhibit Na/H ion exchange in a variety of epithelia and membrane vesicle preparations (19–22), amiloride reduced the increment in sodium absorption ~ 60%. The presence of amiloride did not affect the increment in chloride absorption, but was associated with a P_{CO_2}-induced increase in J^R that was not observed in its absence. These findings suggest that both Na/H and Cl/HCO_3 ion exchange processes are present in the colonic epithelium and respond to alterations in P_{CO_2}.

There are two difficulties with this interpretation of the data. First, amiloride did not completely prevent the CO_2-induced increase in sodium absorption. One possible explanation was that a furosemide-inhibitable sodium absorptive process might have become sensitive to CO_2 (or pH) when Na/H ion exchange was inhibited. We ruled out this possibility by finding that amiloride plus furosemide did not have a greater effect on CO_2-induced changes in sodium absorption than amiloride alone (Table IV). Another explanation for the incomplete amiloride inhibition was methodological: perhaps the concentration of sodium in the bathing solution (140 mM) interfered with amiloride-inhibition of Na/H ion exchange (19, 22, 23). We examined this possibility by substituting choline chloride for sodium chloride to lower the bathing solution sodium concentration to 50 mM. As a result of this change, amiloride completely prevented the P_{CO_2}-induced increase in net sodium absorption. This finding appears to confirm the suggestion that the only sodium absorptive process responsive to CO_2 is Na/H ion exchange. It also may account for the apparent absence of amiloride-inhibitable Na/H ion exchange in other studies of rat distal colon in which a similar bathing solution sodium concentration of 140 mM was used (19, 21, 24, 25).

A second difficulty in concluding that both Na/H and Cl/HCO_3 ion exchange are responsive to CO_2 is our finding that mucosal DIDS at 0.1 and 1 mM did not inhibit CO_2-induced increments in chloride absorption. DIDS and related disulfonic stilbenes are reported to inhibit anion exchange in intact intestinal epithelia and membrane vesicles (26, 27). In recent studies of the rat proximal and distal colon, however, 2 mM mucosal DIDS and 1 mM 4-acetamido-4′-isothiocyanatostilbene-2,2′-disulfonic acid did not alter J^{Na}_{net}, J^{Cl}_{net}, or Isc despite other evidence suggesting that anion exchange was present (18, 28). Similar observations have been made in rabbit ileum (29) and rabbit colon (30). Our results therefore do not absolutely exclude the participation of Cl/HCO_3 ion exchange in the rat distal colon's response to CO_2. As suggested by others, DIDS may not be a sensitive probe for anion exchange under all experimental conditions.

A role for mucosal carbonic anhydrase activity was tested for in a similar way: by inhibiting the enzyme and stimulating absorption with CO_2. In fact, acetazolamide markedly reduced the effect of P_{CO_2} on sodium absorption. This effect of acetazolamide also was observed during the luminal perfusion of the distal colon in intact anesthetized rats (12). The concentration of acetazolamide required to significantly reduce sodium chloride absorption, however, was greater (1 mM) than the concentration required to inhibit the enzyme in broken cell preparations (0.1 mM) (12, 31). Such high concentrations are often needed to inhibit carbonic anhydrase activity in gastrointestinal tissues mounted in Ussing flux chambers (4, 18, 32). This may reflect the moderate water and lipid solubility of acetazolamide and its limited diffusion into tissues (31). Nevertheless, in some tissues, these acetazolamide concentrations may affect ion transport by mechanisms other than carbonic anhydrase inhibition (31). Such nonspecific or toxic effects, however, have not been observed in the rat distal colon (12). The specific effect of carbonic anhydrase inhibition is to alter pHi and as a consequence, electrolyte transport (5, 12, 31, 33). This role for pHi in the colon was suggested in previous studies by the finding that mucosal pHi varied directly with changes in arterial P_{CO_2} and inversely with changes in sodium absorption (11). In the present study, it is thus likely that acetazolamide blunted the effect of P_{CO_2} on colonic absorption by altering the pHi response to P_{CO_2}.

Finally, it was noteworthy that the effect of the plasma HCO_3 concentration on colonic net HCO_3 secretion observed in vivo (3) was not found in the Ussing flux chamber. Only a slightly higher level of J^R was found at a bathing solution HCO_3 concentration of 39 mM (pH 7.65) than at 11 mM (pH 7.14). Although J^R may not always be a reliable measure of net HCO_3 secretion (4, 29), in our study, J^R appeared to reflect HCO_3 transport. For example, J^R increased in response to increases in P_{CO_2} in the presence of either amiloride or acetazolamide, additions that would be expected to cause disproportionate responses in Na/H and Cl/HCO_3 ion exchange. In addition, J^R was zero and did not respond to changes in pH in the absence of bathing solution CO_2 and HCO_3 (Hepes buffer). We believe the difference between the in vivo and in vitro findings may be due to the equivalent HCO_3 concentrations in the mucosal and serosal solutions in the chamber, and the differing HCO_3 concentrations in the plasma and luminal perfusate in vivo. The in vivo results thus may have reflected the effect of a transmucosal HCO_3 concentration gradient on intercellular HCO_3 movement. It is also likely that the relatively large chamber volume and unpaired design of the HCO_3 experiments limited our ability to detect small changes in net

HCO_3 secretion. This is especially relevant in light of the recent finding, using a new in vitro technique, that extracellular HCO_3 concentration apparently does modulate active colonic HCO_3 secretion (9, 34). For these reasons, our conclusions regarding the effects of HCO_3 concentration and various inhibitors on J^R and HCO_3 secretion by the colon must be tentative.

In summary, these studies establish a primary role for CO_2 in regulating active sodium chloride absorption by the rat distal colon. The transport pathways stimulated by increasing P_{CO_2} appear to be Na/H and Cl/HCO_3 ion exchange processes located along the luminal border of colonic epithelial cells. This effect of CO_2 requires mucosal carbonic anhydrase activity to generate the pHi change that mediates the observed changes in electrolyte transport. In general, these findings confirm previous observations, although a separate effect of extracellular HCO_3 concentration on active colonic HCO_3 secretion was not found.

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