Electrogenic Anion Absorption in Rabbit Distal Colon (43351)

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Abstract. **The mechanisms of anion transport in the rabbit distal colon were investigated *in vitro* under short-circuit conditions by examining the effects of transport inhibitors (the stilbene derivatives SITS and DIDS) under a variety of conditions. These agents consistently inhibited $J^{Cl}_{m\text{-}s}$: SITS (10^{-3} *M*) reduced both unidirectional chloride fluxes to the same degree and did not alter J^{Cl}_{net}. In contrast, 10^{-4} *M* DIDS had no effect on $J^{Cl}_{s\text{-}m}$ and had a significant chloride antiabsorptive effect. DIDS had no effect on either tissue cyclic AMP levels or on basal flux of potassium. The effects of SITS and the cyclic AMP-related secretagogue theophylline on I_{sc} were independent. Additionally, there was no significant alteration of intracellular potential difference or apical membrane fractional resistance elicited by SITS during microelectrode impalement of colonic surface epithelial cells. These results suggest a complex mechanism of anion transport in the distal colon, with a component of electrogenic anion absorption inhibited by the stilbenes. The subsequent changes in current, conductance, and chloride fluxes are dependent upon additional, independent anion transport processes. These pharmacologic agents exhibit an antiabsorptive effect, rather than a stimulation of electrogenic chloride secretion.**

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Previous studies have delineated several of the basic aspects of anion transport in the distal colon. Chloride absorption is generally considered to be an electroneutral, Na-independent process mediated by an apical anion exchange mechanism. The basolateral exit step and the regulators of Cl absorption have yet to be fully defined. Much of our understanding of the mechanisms of epithelial ion transport has been gained by utilizing pharmacologic agents as inhibitors of specific transport systems. Although this approach has led to several advances in our understanding, it is fraught with pitfalls if one assumes that a pharmacologic agent has a single action. In this study, we have employed two frequently used transport inhibitors, 4-acetamido-4′-isothiocyanatostilbene 2,2′-disulfonate (SITS) and 4,4′-diisothiocyanatostilbene-2,2′-disulfonic acid (DIDS), to investigate ion transport in the rabbit distal colon *in vitro*. While the disulfonic stilbenes SITS and DIDS are most frequently used as inhibitors of anion exchange (1), they clearly have additional effects (2–4).

Our studies suggest that a component of anion absorption in the distal colon is electrogenic and conductive. The role of this transport system in the overall function of colonic transport remains to be determined.

Materials and Methods

New Zealand White male rabbits (2–3 kg) were fed standard rabbit chow and water *ad libitum*. Rabbits were sacrificed by cervical dislocation, and the distal colon, 10 cm of colon aborad to the anal verge, was removed, opened along its mesenteric border, and rinsed of luminal contents. Before use, tissues were maintained in an ice-cold Ringer solution bubbled with 95% O_2 and 5% CO_2. The serosa and two muscle layers were removed by placing a sheet of colon, serosa up, on a Plexiglas (or acrylic) plate moistened with Ringer solution, making a transverse incision through the muscle layers with a razor blade, and peeling the layers off longitudinally with fine, curved forceps.

Solutions. Several different Ringer solutions were used. The standard Ringer solution contained (in mmol/liter): NaCl, 114; KCl, 5; Na_2HPO_4, 1.65; NaH_2PO_4, 0.3; $CaCl_2$, 1.25; $MgCl_2$, 1.1; $NaHCO_3$, 25; and glucose, 10. The chloride-free Ringer solution was composed of (in mmol/liter): Na gluconate, 114; K gluconate, 5; Mg gluconate, 1.1; Ca gluconate, 1.25;

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$NaHCO_3$, 25; NaH_2PO_4, 0.30; and glucose, 10. The bicarbonate-free Ringer was composed of (in mmol/liter): NaCl, 114; KCl, 5; $MgCl_2$, 1.1; $CaCl_2$, 1.25; NaH_2PO_4, 1.65; Na_2HPO_4, 0.30; glucose, 10; HEPES, 5; and mannitol, 45. The Cl, bicarbonate-free Ringer was composed of (in mmol/liter): Na gluconate, 114; K gluconate, 5.0; Mg gluconate, 1.1; Ca gluconate, 1.25; NaH_2PO_4, 1.65; and Na_2HPO_4, 0.30; and 10 m*M* glucose, 5 m*M* HEPES, and 45 m*M* mannitol. The bicarbonate-free solutions were bubbled with 100% O_2 and adjusted to pH 7.4. Choline was substituted for Na to obtain Na-free solutions.

Electrical and Ion Flux Measurements. Transepithelial potential difference, total conductance (G_t), and short-circuit current (I_{sc}) were measured as described previously (5). Briefly, six pieces of stripped intestinal mucosa were mounted in Ussing chambers (1.12-cm^2 exposed surface area) and bathed with 10 ml of the appropriate Ringer solution on each side. Solutions were circulated by gas lift and maintained at 37°C in water-jacketed reservoirs. Basal electrical parameters were measured after tissue stabilization under short-circuit conditions, generally 45 min after mounting in Ussing chambers. Peak values were used as the electrical response to various agents. If no change in I_{sc} was evident, the I_{sc} and G_t measured 3–5 min after addition of the agent were recorded as the response. Unless otherwise noted, 10^{-4} *M* amiloride was continuously present in the mucosal reservoir.

Ion fluxes were measured as described previously (5). After the tissues had been mounted 45 min, ^{22}Na and ^{36}Cl or ^{42}K were added to either the mucosal (m) or serosal (s) reservoir of the paired tissues (G_t difference of less than 25%). Unidirectional mucosal to serosal and serosal to mucosal fluxes and the net fluxes of ions were calculated from aliquots taken at the beginning and end of an initial 30-min flux period (Period I) and a second 40-min flux period after the addition of the appropriate agent (Period II).

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 was calculated as the difference between oppositely directly unidirectional fluxes of tissue pairs ($\Delta J_{net} = J_{m\rightarrow s} - J_{s\rightarrow m}$). Residual ion flux ($J^R$) represents that portion of I_{sc} not accounted for by Na and Cl fluxes.

K-Depolarization. The effects of membrane potential on the electrical responses were evaluated by determining drug-induced changes in I_{sc} and G_t under conditions in which the voltage and resistance of the basolateral membrane are substantially reduced. Apical and basolateral membrane depolarization was accomplished by elevating serosal K to 100 m*M* (equimolar substitution of K gluconate for Na gluconate) under short-circuit conditions (6). The activity coefficient for K in gluconate Ringer was determined by carefully making solutions with known K gluconate concentrations and then measuring the K activity using a Medica Corp. ion analyzer with a K-selective macroelectrode.

Microelectrode Studies. Studies employing conventional glass microelectrodes to measure voltages across apical and basolateral cell membranes of surface epithelial cells were performed as described previously (6). All impalements were made through the basolateral membrane via a small "window" microdissected through the residual connective tissue in the basolateral membrane. Assurances that the tip of the microelectrode was located in a surface epithelial cell were 3-fold. First, the electrode tip was visually positioned to enter the tissue in a region between crypts using a stereozoom microscope (Olympus); second, the cell hyperpolarized rapidly upon the blocking of I_{sc} by mucosal amiloride; and third, subsequent advancement of the electrode by a few microns caused the tip of the electrode to cross a single resistive barrier and enter the mucosal solution (determined electrically). All experiments were conducted in the standard Ringer solution. Compensation was made for the resistance of the fluid between the voltage-sensing electrodes. Relative step resistances of the apical and basolateral membranes, r^m and r^s, were obtained using intermittent 10 mV bipolar step changes in the ψ^{ms} of 1-sec duration. The voltage divider $\Delta\psi^{mc}/\Delta\psi^{ms}$ was assumed to be equal to the fractional resistance ($f = r^m/[r^m + r^s]$).

Cyclic AMP Assays. Cyclic AMP was measured as described by Rao *et al.* (7) and Steiner *et al.* (8). Stripped epithelia were minced into ≈16-mm^2 pieces, incubated for 20 min at 37°C in 5 ml of Ringer's solution in Erlenmeyer flasks gassed with 95% O_2 and 5% CO_2. No agents were added to the control tissues, while test tissues had either DIDS or forskolin added at 10^{-4} *M* for 2 or 5 min. Tissues were then rapidly placed in cold 10% trichloracetic acid, homogenized with 10 strokes of an Ultra-Turrax Tekmar Tissueizer, and centrifuged at 3000*g* for 20 min. Protein determinations were done on the pellet; the trichloracetic acid was extracted with water-saturated ether and the residual ether was removed from the aqueous phase by heating to 50°C for 30 min. The radioimmunoassay was performed as described by Steiner *et al.* (8).

Statistics. Where appropriate, Student's paired *t* test was used. Otherwise, Student's unpaired *t* test was employed for comparisons between groups.

Materials. The following materials were used: ^{22}Na, ^{42}K, [^3H]mannitol (New England Nuclear), ^{36}Cl (ICN Pharmaceutical, Irvine, CA), amphotericin (Fungizone Intravenous; Squibb, Princeton, NJ), and amiloride (a generous gift from Frank A. Cuter, Merck Sharp & Dohme, West Point, PA). Furosemide (Lasix) was obtained from Hoechst Pharm. The cyclic AMP antibody, [^{125}I]-succinyl cAMP tyrosine methyl ester, and cAMP standards were a generous gift from Jeffrey Harper (University of Texas Medical School, Houston, TX).

The remaining reagents were obtained from Sigma Chemical Co. (St. Louis, MO).

Results

The Effect of SITS on Cl Transport. SITS is a potent inhibitor of anion exchange in red blood cells; however, its effects on vectorial transport, especially in the intestine, have not been well defined. Therefore, we examined the effect of the serosal addition of 1 m*M* SITS on sodium and chloride transport in rabbit distal colon *in vitro* (Table I). The mucosal bath contained amiloride (10^{-4} *M*) to eliminate electrogenic Na absorption. SITS caused a rise in short-circuit current (I_{sc}) over 2–5 min that generally plateaued in 5–7 min and then remained stable. This increase in I_{sc} from −0.14 to 0.93 $\mu Eq \cdot cm^{-2} \cdot h^{-1}$ was highly significant and associated with a concomitant decrease in tissue conductance of 25%, from 3.90 to 2.94 mS cm^{-2}. Neither unidirectional nor net Na fluxes were altered by 1 m*M* SITS; however, both $J^{Cl}_{m\text{-}s}$ and $J^{Cl}_{s\text{-}m}$ decreased significantly. Because the change in $J^{Cl}_{m\text{-}s}$ (−1.59 ± 0.36) was slightly greater than that of $J^{Cl}_{s\text{-}m}$ (−1.19 ± 0.28), J^{Cl}_{net} decreased, but not significantly. Since there were no significant changes in net Na or Cl fluxes, it is not possible to ascribe the alteration in I_{sc} to the flux of a specific ion.

To examine the ionic dependence of the change in I_{sc} induced by SITS, we performed a series of electrical experiments in Cl-free, HCO_3-free, and Cl-HCO_3-free Ringers (Table II). The increase in I_{sc} in normal and HCO_3-free Ringer was the same, approximately 32 $\mu A/cm^2$. In Cl-free solutions the electrical response was reduced 45%. In the absence of both Cl and HCO_3, the SITS-induced change in I_{sc} was abolished. Thus, the electrical response to SITS is anion dependent, with HCO_3 being capable of at least partially substituting for Cl. Because of the difference in concentrations of the respective anions (i.e., 25 m*M* HCO_3 vs 121 m*M* Cl) used in these experiments, it is difficult to determine the relative affinities for the SITS-sensitive transport pathway. Addition of SITS to the mucosal bath did not elicit an electrical response. No flux studies were performed with mucosal SITS. The etiology of the higher basal currents in the Cl-free and Cl-free-HCO_3-free conditions is unclear, but may be related to altered cation absorption (9).

The effect of SITS is Na dependent (Fig. 1). Bilateral choline substitution for Na significantly blunts the electrogenic response to SITS. To determine the "sidedness" of this effect, we performed either mucosal or serosal Na substitution. Although it appears that elimination of serosal Na may have a greater effect, the difference between the two unilateral substitutions was not statistically significant. Thus, although there is a clear Na dependence, these data do not allow us to separate a specific mucosal or serosal effect.

Acetazolamide Modification of the SITS Effect. To determine the role of endogenous bicarbonate, we examined the effect of SITS after pretreatment with acetazolamide (10^{-5} *M*) (Table III). SITS decreased both unidirectional Cl fluxes equally, resulting in no change in net Cl transport. Interestingly, there was no significant change in I_{sc} under these conditions.

Effect of SITS on Paracellular Fluxes. Because SITS elicited a decrease in both G_t and unidirectional Cl fluxes, we examined the effect of SITS on the movement of the extracellular marker mannitol. Under control conditions, $J^{mannitol}_{s\text{-}m}$ was 0.013 ± 0.004 $\mu mol \cdot cm^{-2} \cdot hr^{-1}$. After SITS, the flux was essentially unchanged (0.015 ± 0.005 $\mu mol \cdot cm^{-2} \cdot hr^{-1}$ [$n = 4$]). Additionally, $J^{Na}_{s\text{-}m}$ was unchanged by either SITS or DIDS (see Tables I and III). These observations suggest that the stilbenes do not have a generalized effect on paracellular pathways.

DIDS Inhibits Cl Absorption. We examined the effect of a second stilbene derivative, DIDS (10^{-4} *M*), on ion transport in distal colon (Table IV). DIDS elicited a greater increase in I_{sc} (2.03 ± 0.67 $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$) than did 1 m*M* SITS (1.07 ± 0.18 $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$), but a less dramatic decrease in conductance. There were no changes in Na fluxes after exposure to DIDS. Like SITS, DIDS produced a significant decrease in $J^{Cl}_{m\text{-}s}$; however, in contrast to SITS, $J^{Cl}_{s\text{-}m}$ was unchanged by DIDS, so that net Cl secretion was elicited. The changes in J^{Cl}_{net} and I_{sc} were essentially identical, indicating that the increase in short-circuit current could be accounted for by the alteration of Cl fluxes. Thus, the pattern of response to the two stilbenes was different: SITS decreased both unidirectional fluxes, whereas DIDS inhibited $J^{Cl}_{m\text{-}s}$ alone.

Basolateral Membrane Depolarization. In an attempt to clarify the SITS-sensitive mechanisms of transport, we selectively depolarized the basolateral membrane by increasing $[K]_s$ to 100 m*M* (63 m*M* activity),

Table I. Effect of SITS on Ion Transport in Distal Colon[a]

	J^{Na} m-s	$J^{Na}_{s\text{-}m}$	net	J^{Cl} m-s	$J^{Cl}_{s\text{-}m}$	net	I_{sc}	G_t
Control	1.3 ± 0.2	1.2 ± 0.2	0.2 ± 0.2	3.8 ± 0.5	3.3 ± 0.3	0.5 ± 0.4	−0.14 ± 0.06	3.9 ± 0.3
SITS (10^{-3} *M*)	1.5 ± 0.2	1.2 ± 0.1	0.3 ± 0.1	2.2 ± 0.1	2.2 ± 0.1	0.1 ± 0.2	0.93 ± 0.22	2.9 ± 0.3
P	NS	NS	NS	0.005	0.025	NS	0.005	0.001

[a] Results are expressed in mean $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$ ± SE, except for G_t, $mS \cdot cm^{-2}$ for seven animals. Control represents a basal flux period with amiloride (10^{-4} *M*) present in the mucosal reservoir. A second flux period was performed with SITS added to the serosal reservoir.

Table II. Ionic Dependence of SITS[a]

	I_{sc}			G_t		
	AMIL	AMIL + SITS	*P*	AMIL	AMIL + SITS	*P*
NL Ringer (7)[b]	−4.2 ± 2.8	28.2 ± 6.7	0.001	3.90 ± 2.50	2.94 ± 0.25	0.0002
Cl-free (7)	31.3 ± 3.5[c]	44.2 ± 3.5	0.0005	2.56 ± 0.51[c]	3.08 ± 0.71	NS
HCO_3-free (6)	−9.4 ± 5.2	19.8 ± 4.4	0.0009	3.87 ± 0.56	3.32 ± 0.32	NS
CL-HCO_3-free (6)	19.6 ± 1.3[c]	20.8 ± 1.7	NS	2.69 ± 0.44[c]	3.37 ± 0.72	NS

[a] Results are expressed in $\mu A \cdot cm^{-2}$ (I_{sc}) and $mS \cdot cm^{-2}$ (G_t). *P*-values refer to pair-matched controls, comparing tissues before and after addition of SITS.
[b] Number of colons studied.
[c] $P < 0.05$ vs N1 Ringer (unpaired *t* test).

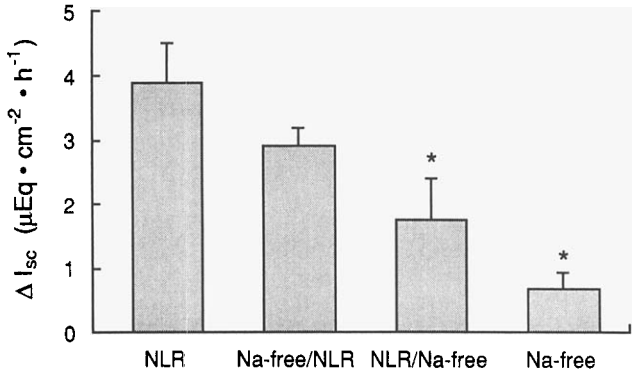

Figure 1. [Na] modification of SITS response. The increase in I_{sc} was measured in response to 10^{-3} *M* serosal SITS in distal colon bathed in normal Ringer bilaterally (NLR), choline substitution for Na either only on the mucosal side (Na-free/NLR), only on the serosal side (NLR/Na-free), or bilaterally Na-free. For each group, $n = 7$. *$P < 0.05$ vs NLR, paired *t* test. There was no significant difference between Na-free/NLR and NLR/Na-free.

which reduces the voltage across the basolateral membrane to approximately −10 mV (S. M. Thompson, unpublished observation) and markedly reduces its resistance (10). Such a reduced potential difference would decrease the driving forces for electrogenic anion exit across the basolateral membrane. Under these conditions, SITS increased I_{sc} by 0.35 ± 0.07 $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$, one third of that found in nondepolarized tissues, but did not significantly alter G_t. Thus, K depolarization substantially reduced the electrical response to SITS, consistent with inhibition of an anion-conductive pathway across the basolateral membrane.

Amphotericin Modifies the Effects of SITS. In a parallel set of experiments, we added amphotericin B (15 μg/ml) to the normal Na Cl Ringer in the mucosal bath. This ionophore has been shown previously to functionally eliminate the apical membrane (at least of surface epithelial cells) as a barrier to cation transport in this tissue and to depolarize the epithelium. Because amiloride does not block Na transport in the presence of amphotericin, electrical responses to SITS may not accurately reflect changes in ion transport; therefore, the effects of SITS on transepithelial Cl fluxes were measured (Table V).

Treatment with amphotericin reduced both J^{Cl}_{ms} and J^{Cl}_{sm} (Table V), compared with untreated tissues (Table I). SITS decreased J^{Cl}_{ms} further; the percentage of decrease was identical with that found in the absence of amphotericin (−45%). Thus, while amphotericin considerably reduced the magnitude of J^{Cl}_{ms}, the relative effects of SITS on this flux were unchanged. In contrast, under control conditions, SITS decreased J^{Cl}_{sm} by 30%, whereas in the presence of amphotericin, SITS had little effect on $J^{Cl}_{s\text{-}m}$ (+5%). I_{sc} increased significantly. Thus, amphotericin did not inhibit, and perhaps enhanced, the response to SITS.

Interactions with Theophylline. Theophylline el-

Table III. Acetazolamide Modification of SITS-Induced Transport Changes[a]

	J^{Na}			J^{Cl}			I_{sc}	G_t
	m-s	s-m	net	m-s	s-m	net		
ACET 10^{-5}	2.9 ± 0.4	1.6 ± 0.3	1.4 ± 0.3	4.8 ± 0.5	4.8 ± 0.8	0.0 ± 0.4	1.2 ± 0.1	4.4 ± 0.4
SITS 10^{-4}	2.7 ± 0.3	1.6 ± 0.3	1.0 ± 0.3	3.4 ± 0.4	3.6 ± 0.6	−0.1 ± 0.3	1.5 ± 0.2	3.7 ± 0.2
P	NS	NS	NS	<0.007	<0.031	NS	NS	<0.018

[a] Results are expressed in mean $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$ ± SE, except for G_t ($mS \cdot cm^{-2}$) for five animals. Amiloride was not present in these studies. During the first flux period, acetazolamide (10^{-5} *M*) was present in both reservoirs. During the second flux period, with acetazolamide still present, SITS was added to the serosal chamber.

Table IV. Effect of DIDS on Ion Transport in Distal Colon[a]

	J^{Na}			J^{Cl}_{s-m}			I_{sc}	G_t
	m-s	s-m	net	m-s	s-m	net		
Control	1.6 ± 0.4	1.4 ± 0.3	0.2 ± 0.3	4.4 ± 0.6	3.9 ± 0.4	0.5 ± 0.5	0.54 ± 0.30	5.0 ± 0.3
DIDS (10^{-3} *M*)	1.8 ± 0.3	1.4 ± 0.2	0.4 ± 0.2	2.6 ± 0.3	4.1 ± 0.4	−1.5 ± 0.5	2.62 ± 0.76	4.7 ± 0.4
P	NS	NS	NS	0.012	NS	0.011	0.034	NS

[a] Results are expressed in mean $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$ ± SE, except G_t ($mS \cdot cm^2$) for six animals. Control represents a basal flux period with amiloride (10^{-4} *M*) present in the mucosal reservoir. A second flux was performed with DIDS (10^{-4} *M*) added to the serosal reservoir.

Table V. Amphotericin Modification of the Effect of Transport Inhibitors[a]

	J^{Cl}			I_{sc}	G_t
	m-s	s-m	net		
AMPHO	1.7 ± 0.2	1.8 ± 0.2	−0.1 ± 0.2	0.87 ± 0.22	4.0 ± 0.6
AMPHO + SITS	1.0 ± 0.1	2.0 ± 0.3	−1.0 ± 0.2	2.49 ± 0.35	4.4 ± 0.9
P	0.03	NS	0.0006	0.0002	NS

[a] Results are expressed in mean $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$ ± SE, except for G_t ($mS \cdot cm^{-2}$). Prior to an initial flux period, amphotericin (15 μg/ml) was added to the mucosal reservoir. Prior to a second flux period, SITS (10^{-3} *M*; n = 6) was added to the serosal reservoir.

evates intracellular cyclic AMP and causes electrogenic Cl secretion (10). We examined the interaction of this classic secretagogue with the electrical effects of furosemide and SITS (Table VI). Pretreatment with SITS did not alter the increase in I_{sc} induced by theophylline, indicating that the stilbene did not block cAMP-mediated secretion. This is in contrast to furosemide, which blocked over two thirds of the theophylline-induced increase in I_{sc}. This latter finding is consistent with previous observations demonstrating that inhibition of the Na-K-2Cl entry step across the basolateral membrane will blunt cAMP-mediated secretion.

Furosemide added after the tissue had been exposed to theophylline caused a significant decrease in I_{sc} compared with control conditions, in which it elicited a modest increase in I_{sc}. Again, the decrease in I_{sc} secondary to furosemide may be ascribed to inhibition of Na-K-2Cl entry. SITS added after theophylline actually caused a greater increase in I_{sc} than under control conditions (2.59 ± 0.54 vs 1.40 ± 0.17 $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$, $P < 0.05$), indicating that cAMP may enhance the SITS-induced electrical response. Either cAMP itself or the stimulation of active secretion may be involved in altering the stilbene-sensitive transport process.

Table VI. Interaction of Theophylline with Transport Inhibitors[a]

Control	Treatment	*n*	ΔI_{sc}	Significance (*P*)
AMIL + SITS	THEO	5	2.98 ± 0.45	NS
AMIL	THEO	7	3.15 ± 0.33	
AMIL + FUROS	THEO	4	0.79 ± 0.32	<0.05
AMIL	THEO	4	2.83 ± 0.50	
AMIL + THEO	SITS	7	2.59 ± 0.54	<0.05
AMIL	SITS	7	1.40 ± 0.17	
AMIL + THEO	FUROS	4	−1.08 ± 0.23	<0.01
AMIL	FUROS	4	0.42 ± 0.12	

[a] ΔI_{sc} represents the peak change in I_{sc} (expressed in mean $\mu Eq \cdot cm^{-2} \cdot hr^{-1}$ ± SE) elicited by the specific treatment. Additions were made in the following manner: mucosal, amiloride (10^{-4} *M*); serosal, theophylline (10^{-2} *M*); SITS (10^{-4} *M*); and furosemide (10^{-3} *M*).

SITS: Microelectrode Studies. To evaluate the effect of SITS on the electrical properties of the apical and basolateral membranes of the colonic epithelium, we selectively impaled surface epithelial cells and recorded ψ^{mc}, the electrical potential difference across the apical membrane, and the fractional resistance ($f = \Delta\psi^{mc}/\Delta\psi^{ms}$), which is assumed to be equal to that portion of the total cellular resistance accounted for by the apical membrane ($r^m/(r^m + r^s)$). The data (Table VII) demonstrate that under both normal conditions and in the presence of mucosal amiloride, SITS increased I_{sc} and decreased G_t, but in neither case did SITS significantly affect the ψ^{mc} or f. In individual experiments, it was clear that ψ^{mc} remained constant, despite large increases in I_{sc} induced by SITS, and that in these same cells, ψ^{mc} rapidly hyperpolarized upon mucosal addition of amiloride. These results suggest, therefore, that SITS does not elicit its transepithelial response via an effect on the surface epithelial cells.

DIDS Does not Stimulate Cyclic AMP. We examined the effect of DIDS on cAMP production in colonic mucosa. Control epithelium averaged 2.00 ± 0.08 pmol cAMP/mg protein. Forskolin (10^{-4} *M*) produced a 10- to 20-fold increase in tissue cyclic AMP, while DIDS (10^{-4} *M*) failed to increase nucleotide levels above baseline (Table VIII). Thus, the changes in Cl fluxes induced by DIDS are not a consequence of changes in cAMP content.

Table VII. Effects of SITS on Ψ^{mc} and Fractional Resistance

Treatment	I_{sc}	G_t	Ψ^{mc}	F
A Control	27.9 ± 10.5	4.1 ± 0.5	−34.2 ± 3.5	0.73 ± 0.07
SITS ($n = 5$)	46.9 ± 10.8[b]	3.6 ± 0.3	−34.2 ± 3.5	0.73 ± 0.07
B SITS	49.5 ± 13.6	3.3 ± 0.3	−34.5 ± 4.6	0.74 ± 0.08
SITS + AMIL	15.3 ± 2.6[b]	3.1 ± 0.3	−46.3 ± 6.4[c]	0.84 ± 0.11

[a] Experimental protocol involved recording continuously from a single surface epithelial cell impaled with a microelectrode while bathing solutions were changed. Results are expressed as mean ± SE, for I_{sc}, in $\mu A \cdot cm^{-2}$; Ψ^{mc}, MV.
[b] $P = 0.01$ vs pair-matched controls.
[c] $P = 0.03$.

Table VIII. Effect of DIDS on Epithelial cAMP[a]

Min	Forskolin	DIDS
2 min	10.03 ± 1.18	0.97 ± 0.05
5 min	21.29 ± 3.94	0.99 ± 0.04

[a] Results are expressed as a stimulation index, which represents the ratio of cAMP in treated tissue compared with controls. An index of 1.0 represents no stimulation. Both DIDS and forskolin were used at a 10^{-4}-*M* concentration. Results are for five experiments.

K Fluxes Are not Altered by DIDS. To determine whether the electrical response to stilbenes may be related to a change in K transport, we examined the effects of DIDS on K fluxes in the distal colon. (Table IX) DIDS elicited the expected increase in I_{sc}, but had no discernible effect on either unidirectional or net K transport. Thus, the DIDS-induced changes in I_{sc} cannot be accounted for by changes in K absorption.

Discussion

Chloride absorption in the rabbit distal colon has been generally modeled to occur via an electroneutral anion exchange process at the apical membrane and a poorly defined, but probably electroneutral, basolateral exit step, such as a KCl cotransport process (11). Transepithelial chloride absorption is considered to be electrically silent. Therefore, evidence of an electrogenic anion absorptive process is of particular significance.

In attempting to delineate the mechanisms of anion transport in the rabbit distal colon, we used two well-recognized transport inhibitors: SITS and DIDS. The observed changes in Cl fluxes are complex and are not consistent with simple anion exchange inhibition or blockage of basolateral Na-K-2Cl entry. Changes in I_{sc} elicited by these agents suggest that the transporters affected are electrogenic, rather than electroneutral. The decreases in G_t are consistent with an effect on a conductive pathway, although it is unlikely to be either a simple channel or an alteration in paracellular conductance. Whereas the process of electrogenic Cl secretion is well established, there are few previous reports suggesting electrogenic anion absorption. In the present study, the consistent antiabsorptive effect of the stilbenes, coupled with changes in I_{sc} and G_t, supports the hypothesis of electrogenic anion absorption. Anion substitution data suggested that both HCO_3 and chloride may share this transport system (see below).

Despite the variable response in net Cl transport and I_{sc} among different agents and under differing conditions, a certain reproducible pattern emerges. SITS and DIDS consistently decreased $J^{Cl}_{m\text{-}s}$; this occurred under all experimental conditions and suggests a common antiabsorptive effect shared by the two drugs. The variability in net Cl flux between these agents is related to the different patterns of change in $J^{Cl}_{s\text{-}m}$. When $J^{Cl}_{s\text{-}m}$ and $J^{Cl}_{m\text{-}s}$ are reduced equally, e.g., SITS in normal Ringer, there is no significant change in net Cl transport. When $J^{Cl}_{s\text{-}m}$ is unchanged, in conjunction with a reduced $J^{Cl}_{m\text{-}s}$, e.g., DIDS or SITS in amphotericin-treated tissues, a clear secretory effect is elicited.

Thus, the variable responses are probably due to differing effects on two or more transport systems. In the basal state, rabbit colon actively and electrogenically absorbs and secretes both cations and anions simultaneously, so that the I_{sc} is a composite of the individual transport processes. SITS and DIDS appear to abolish an electrogenic anion absorptive process. The effect of blocking a negative component of the I_{sc} due to anion

Table IX. Effect of DIDS on Potassium Fluxes[a]

	m-s	s-m	net	I_{sc}	G_t
Control	0.39 ± 0.05	0.49 ± 0.11	−0.10 ± 0.12	0.26 ± 0.16	3.8 ± 0.3
DIDS (10^{-4} *M*)	0.47 ± 0.07	0.53 ± 0.22	−0.11 ± 0.22	1.97 ± 0.32[b]	4.2 ± 0.3

[a] Sequential fluxes are performed in distal colon first under basal conditions and then after exposure to serosal DIDS ($n = 4$).
[b] $P = 0.005$.

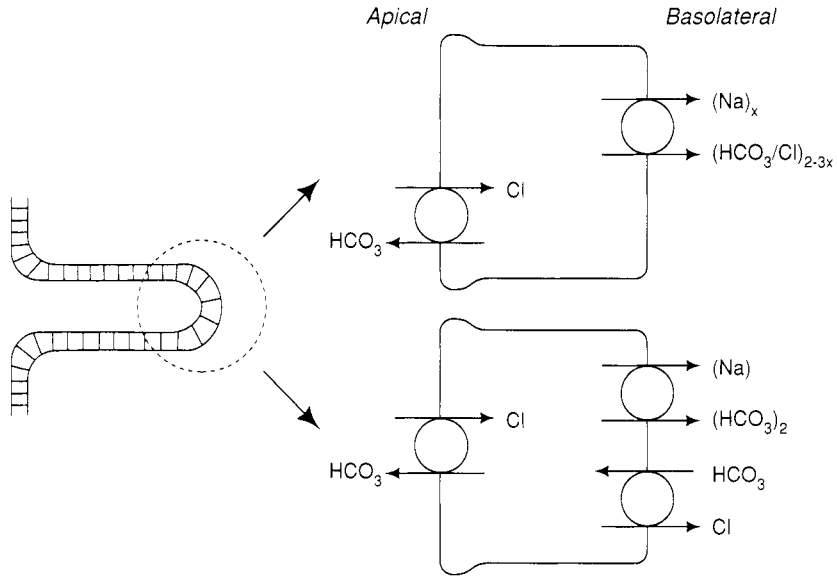

Figure 2. Potential site of stilbene effect (see text). Several observations have suggested a crypt, rather than villous, localization. Because the response occurs with serosal, rather than mucosal, addition of stilbenes, the transporter process is more likely on the basolateral border. K depolarization experiments are consistent with this site. Ion substitution experiments suggest a dependency on Na, Cl, and HCO_3. The stilbene-sensitive process may represent a symport carrier with variable affinity for Cl and HCO_3. Alternatively, it may represent two carrier systems involving an anion exchange system and a Na-HCO_3 cotransport.

absorption is, therefore, to increase I_{sc} and to unmask the positive current due to anion secretion. Consistent with this is the finding that DIDS, which gives the largest increase in I_{sc}, decreases $J^{Cl}_{m\text{-}s}$, but has no effect on $J^{Cl}_{s\text{-}m}$.

Previous investigators have concluded that the alterations in Cl transport induced by stilbenes were due to an increase in prostaglandin-induced electrogenic Cl secretion (4). DIDS induced a bumetanide- and indomethacin-inhibitable increase in I_{sc} and chloride secretion. Several of our observations suggest that this is not the case. Electrogenic Cl secretion in distal colon generally increases both $J^{Cl}_{s\text{-}m}$ and G_t. Neither of these occurred with the stilbenes; instead $J^{Cl}_{m\text{-}s}$ decreased and G_t either decreased or remained constant. There was no observable increase in tissue cyclic AMP, as would have been expected with prostaglandin-mediated secretion. Exposure of the epithelium to theophylline, which increases tissue cyclic AMP, did not block the effect of SITS, but rather may have potentiated it. Microelectrode studies have demonstrated previously that cAMP-mediated colonic secretion is characterized by depolarization of ψ^{mc} and a decrease in fractional resistance in both surface and crypt cells (12). However, Emmer and Duffey (13) found that DIDS caused a hyperpolarization of ψ^{mc} and an increase in fractional resistance in both surface and crypt cells, results opposite those of cAMP-mediated secretion. Thus, their study suggests that disulfonic stilbenes alter Cl transport by a very different mechanism than that associated with cAMP. Although these observations do not provide direct evidence for inhibition of electrogenic Cl absorption by SITS or DIDS, they are not consistent with stimulation of Cl secretion.

Several different techniques were employed to localize the stilbene-sensitive transport processes. The amphotericin and microelectrode experiments suggest that this transporter resides primarily in the crypt cells. Because amphotericin permeabilizes the apical membrane of the surface epithelium and collapses the electrochemical gradient, the persistence of the SITS response under these conditions implies an amphotericin-insensitive site, i.e., crypt cells. We could not demonstrate any electrophysiologic response to SITS in surface cells, despite definite changes in transepithelial electrical parameters. Again, this could be due to the fact that SITS primarily affects crypt cells.

Depolarization of the basolateral membrane (accomplished by increasing serosal K) significantly blunted the electrical response to SITS. This response is consistent with an inhibition of an electrogenic exit step across the basolateral membrane, rather than an apical entry pathway. Thus, these sets of experiments provide circumstantial evidence localizing the stilbene-sensitive transporter to the basolateral membrane of crypt cells.

The ion substitution experiments suggest that the change in I_{sc} may be linked to both Cl and HCO_3. The increase in I_{sc} is attenuated in Cl-free solutions and totally inhibited in Cl-HCO_3-free solutions (Table II). Acetazolamide blocks endogenous bicarbonate production and inhibits electroneutral chloride transport in rat colon (14). In the presence of acetazolamide, SITS decreased both unidirectional fluxes of chloride, suggesting that the two drugs have independent effects on

Cl transport. However, acetazolamide did attenuate the electrical response to SITS, implying that this may be a bicarbonate-related process. Sodium clearly has a permissive role in this process, as demonstrated by the Na-substitution experiments, although the "sidedness" of this effect will require further clarification because of the multiplicity of transporters that may be coupled to Na in a secondary or tertiary manner.

It is possible, given the present data, to propose two preliminary alternative models of the stilbene-sensitive transport process: either (i) a $(Na)_x$ $(anion)_{2-3x}$ basolateral carrier that will accept both Cl and HCO_3, or (ii) a set of two transporters including a $(Na)_x(HCO_3)_{2x}$ exit step that would function in close association with a $Cl:HCO_3$ exchanger. In the latter model, HCO_3 would recycle across the basolateral membrane. These models are clearly speculative at the present time (Fig. 2). In other epithelia, stilbenes have been shown to inhibit both of these transporters. The relatively nonspecific effects of SITS and DIDS on multiple transporters make it imperative to interpret these data cautiously.

Initially, anion transport in the distal colon was described as being electroneutral, mediated by an apical $Cl:HCO_3$ exchange (15). However, the basolateral exit step for anions has not been clearly determined. Recent studies have suggested several possible anion transport mechanisms, many of which are sensitive to stilbenes and furosemide (15–20). The basolateral membrane quite clearly has a multiplicity of possible transport systems; further studies will be required to resolve the complexity of these systems.

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