Enteropathogenic E. coli attenuates secretagogue-induced net intestinal ion transport but not Cl− secretion

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Hecht, Gail, and Athanasia Koutsouris. Enteropathogenic E. coli attenuates secretagogue-induced net intestinal ion transport but not Cl− secretion. Am. J. Physiol. 276 (Gastrointest. Liver Physiol. 39): G781–G788, 1999.—Enteric bacterial pathogens often increase intestinal Cl− secretion. Enteropathogenic Escherichia coli (EPEC) does not stimulate active ion secretion. In fact, EPEC infection decreases net ion transport in response to classic secretagogues. This has been presumed to reflect diminished Cl− secretion. The aim of this study was to investigate the influence of EPEC infection on specific intestinal epithelial ion transport processes. T84 cell monolayers infected with EPEC were used for these studies. EPEC infection significantly decreased short-circuit current (Isc) in response to carbachol and forskolin, yet 36Cl− efflux studies revealed no difference in Cl− channel activity. There was also no alteration in basolateral K+ channel or Na+-K+ -2Cl− cotransport activity. Furthermore, net 36Cl− flux was not decreased by EPEC. No alterations in either K+ or Na+ transport could be demonstrated. Instead, removal of basolateral bicarbonate from uninfected monolayers yielded an Isc response approximating that observed with EPEC infection, whereas bicarbonate removal from EPEC-infected monolayers further diminished Isc. These studies suggest that the reduction in stimulated Isc is not secondary to diminished Cl− secretion. Alternatively, bicarbonate-dependent transport processes appear to be perturbed.

infectious diarrhea; enteric pathogens; bicarbonate

THE PARADIGM TYPICALLY associated with diarrhea that results from infection by an enteric pathogen is one of Cl− secretion or, somewhat less commonly, inhibition of Na+ and Cl− absorption. As more effort has been invested into studying the interactions between infectious agents and their target host cells, previously unrecognized mechanisms of pathogenesis have been defined. For example, it is now clear that several enteric bacterial pathogens and/or their toxins alter tight junction permeability of intestinal epithelia (9). Some, such as toxins A and B of Clostridium difficile, appear to induce this physiological function by affecting the actin cytoskeleton and specific tight junction proteins via inactivation of Rho (10, 11, 21). Others may directly affect tight junction structure, such as zonula occludens toxin of Vibrio cholera (8). Still others, such as enteropathogenic Escherichia coli (EPEC), alter tight junction permeability by activating specific signaling pathways (24). EPEC attachment to its host target cell induces the phosphorylation of myosin light chain, thereby stimulating contraction of the perijunctional actomyosin ring and opening tight junctions (27). Perturbation of tight junction barrier function may interfere with vectorial transport by allowing back diffusion and equilibration of electrochemical gradients to occur. This isolated alteration in intestinal epithelial physiology, however, is unlikely to be sufficient to account for the resultant diarrhea associated with EPEC.

EPEC is a most interesting pathogen as it is neither invasive nor toxigenic. Yet somehow by intimately attaching to its host cell, it activates a number of signal transduction pathways that then presumably translate into alterations in intestinal epithelial function, in turn resulting in diarrhea. Interestingly, we and others have previously reported (23, 26) that cultured intestinal epithelial T84 cell monolayers infected with EPEC exhibit an attenuation in net ion transport, measured as short-circuit current (Isc), in response to classic secretagogues such as forskolin and carbachol. This observation, presumed to reflect decreased Cl− secretion, has not been examined in detail. The aim of this study was to investigate the effects of EPEC on intestinal epithelial transport processes with specific attention to Cl− secretion. For these investigations, we have employed the well-characterized human intestinal epithelial cell line, T84, and a commonly used clinical isolate of EPEC, strain E2348/69.

METHODS

Culture of T84 cells. T84 cells were kindly provided by Dr. Kim Barrett (University of California, San Diego, CA). Passages 25–40 were used for these studies. Cells were cultured as previously described (18) in a 1:1 mixture of Ham’s F-12 and DMEM supplemented with 6% newborn calf serum.

Bacterial cultures. EPEC strain E2348/69, a gift from Dr. James Kaper (Center for Vaccine Development, University of Maryland, Baltimore, MD), was initially grown overnight in Luria-Bertani broth. On the day of experimentation, 30 µl of EPEC culture were transferred to 1 ml of serum- and antibiotic-free T84 cell medium and grown to early or midlog growth phase. T84 cell monolayers were then infected with 2 × 10⁵ colony-forming units (cfu) of EPEC for 1 h. Nonadher-
ent bacteria were then removed by washing, and incubation proceeded for another 1–2 h. EPEC strain UMD864, an espB deletion mutant, was provided by Dr. Michael Donnenberg (Section of Infectious Diseases, University of Maryland, Baltimore, MD).

Electrophysiological studies. A simplified apparatus for measuring electrophysiological parameters previously described by Madara et al. (16) was used for these studies. Isc in response to carbachol (10^{-4} M) or forskolin (10^{-6} M) was determined. Resistance was calculated using Ohm's law (V = I/R, where V is voltage, I is current, and R is resistance) by determining voltage produced in response to the passage of 25 µA of current. All experiments were performed in Ringer solution containing (in mM) 144 NaCl, 5 KCl, 1.65 Na_2HPO_4, 0.3 NaH_2PO_4, 25 NaHCO_3, 1.1 MgSO_4, 1.25 CaCl_2, and 5 glucose unless indicated otherwise. For select experiments, specific ion-free buffers were used. Na^+ -free buffer contained (in mM) 116 choline chloride, 5 KCl, 1.65 K_2HPO_4, 0.3 KH_2PO_4, 25 choline HCO_3, 1.1 MgSO_4, 1.25 CaCl_2, and 10 glucose. HEPES-phosphaté-buffed Ringer solution (HPBR) was used for HCO_3^-free studies and contained the following (in mM): 135 NaCl, 5 KCl, 3.33 Na_2HPO_4, 0.83 Na_2HPO_4, 1 CaCl_2, 1 MgCl_2, 5 HEPES, and 10 glucose.

Experiments examining the effect of Na^+ or HCO_3^- removal on secretagogue-stimulated I sc were performed as follows: T84 monolayers were infected with EPEC for 1 h, after which nonadherent organisms were removed by washing. Incubation was continued for an additional 1–2 h. Select buffers were added to either the apical or basolateral reservoir as indicated and incubated for 1 h. I sc was then measured in response to secretagogues.

Results

Effect of EPEC on baseline and secretagogue-stimulated I sc. Confluent T84 monolayers (0.33 cm^2) were infected with 2 × 10^7 midlog growth phase EPEC, and I sc was measured at 15- to 30-min intervals for up to 6 h. At no point in time did EPEC stimulate the I sc above baseline (data not shown). To determine whether EPEC infection influenced the I sc response to secretagogues, uninfected and infected monolayers were challenged with either the Ca^{2+}-mediated secretagogue carbachol (10^{-4} M) or the cAMP-mediated secretagogue forskolin (10^{-6} M). As shown in Fig. 1, EPEC infection significantly decreased I sc in response to both carbachol and forskolin compared with uninfected controls. Although EPEC has been previously demonstrated to increase tight junction permeability, that attenuation in I sc could not be attributed to alterations in resistance was shown in two ways. First, the changes in secretagogue-induced I sc could be demonstrated before the EPEC-associated decrease in resistance occurred (Fig. 2). Second, the EPEC mutant UMD864, a deletion of the espB gene necessary for the activation of several host...
signaling pathways, does not alter transepithelial resistance (+20 ± 8 vs. −6 ± 5% change in resistance for UMD864 vs. control), yet it still causes the observed attenuation in forskolin-stimulated I_{sc} (90 ± 4 vs. 62 ± 4 μA/cm² for control and UMD864, respectively). Sterile bacterial supernatants had no effect on secretagogue-stimulated I_{sc} (data not shown), suggesting that a soluble factor is not responsible.

Diminished Cl⁻ secretion does not account for the decrease in secretagogue-induced I_{sc}. T84 monolayers have been widely used as a model cell line in which to study Cl⁻ secretion (1). Because EPEC has been shown to diminish the I_{sc} response in T84 monolayers challenged with secretagogues, it has been presumed that decreased Cl⁻ secretion is responsible. This hypothesis, however, has not been directly tested. Electrogenic Cl⁻ secretion requires the coordinated activity of several ion transporters. These include the apical Cl⁻ channel and basolateral Na⁺-K⁺-ATPase, Na⁺-K⁺-2Cl⁻ cotransporter, and K⁺ channels. Our approach, therefore, was to systematically evaluate the effect of EPEC on the transport components that contribute to apical Cl⁻ secretion.

Cl⁻ channel activity was investigated first by performing ¹²⁵I efflux studies in uninfected and infected T84 cell monolayers. This approach has been previously validated as an acceptable method for examining apical Cl⁻ channel activity in T84 cells (25). Surprisingly, there was no significant difference in ¹²⁵I efflux from uninfected and EPEC-infected monolayers stimulated with either carbachol (0.068 ± 0.005 vs. 0.075 ± 0.014 for control and EPEC-infected monolayers, respectively) or forskolin (Fig. 3). It was suggested previously that the diminished I_{sc} response in EPEC-infected monolayers might be secondary to inhibition of the basolateral Na⁺-K⁺-2Cl⁻ cotransporter, known to be regulated by the actin (19) and myosin (15) cytoskeleton. No difference in cotransporter activity, however, was detected as assessed by ⁸⁶Rb uptake studies in response to carbachol (9.6 ± 1.6 vs. 8.1 ± 0.7 nmol K⁺·min⁻¹·mg protein⁻¹ for control and EPEC-infected monolayers, respectively; n = 6; P = 0.34) or forskolin (Fig. 4A). Similarly, no diminution in basolateral K⁺ channel activity in EPEC-infected monolayers was seen as determined by basolateral ⁸⁶Rb efflux studies (Fig. 4B). Taken together, these data demonstrate that each transport component required for apical Cl⁻ secretion to occur is fully functional in EPEC-infected monolayers.

To confirm that EPEC infection did not alter net Cl⁻ secretion, unidirectional ³⁶Cl⁻ flux studies were performed in modified Ussing chambers. These data are presented in Table 1. In control monolayers stimulated with forskolin, the I_{sc} predicted by the net secretion of Cl⁻ is ~40% less than the corresponding measured I_{sc}. In EPEC-infected monolayers, the predicted and measured I_{sc} more closely corresponded, showing that only 17% of I_{sc} is not attributable to Cl⁻ secretion. There was no significant difference in forskolin-stimulated, unidirectional, or net movement of ³⁶Cl⁻ across control and

![Fig. 2. Alterations in barrier function do not account for diminished I_{sc}. A: effect of EPEC on transepithelial electrical resistance after 1, 2, and 3 h of infection. Even after 3 h of infection with EPEC, there is no significant change in resistance. B: EPEC-induced diminution in forskolin-stimulated I_{sc} is not related to decreased resistance. At 1, 2, and 3 h postinfection with EPEC when resistance remains unchanged, a 25–35% decrease in I_{sc} is observed. For A and B, n = 6 and P < 0.01.](http://ajpgi.physiology.org/)

![Fig. 3. EPEC infection does not alter Cl⁻ channel activity. Control and infected T84 cell monolayers were loaded with ¹²⁵I as described in METHODS. ¹²⁵I efflux was then determined at 2-min intervals following stimulation with forskolin (n = 10–12, P > 0.05 for all time points).](http://ajpgi.physiology.org/)

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**EPEC DECREASES NET ION TRANSPORT IN INTESTINAL CELLS**

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Neither stimulation of apical K⁺ secretion nor inhibition of Na⁺ absorption accounts for diminished I_{sc} in EPEC-infected monolayers. Because diminished Cl⁻ secretion did not explain the attenuated I_{sc} response in EPEC-infected monolayers, alternative explanations were explored. First, the possibility that apical K⁺ secretion might be stimulated, thus blunting measured I_{sc}, was examined. To test this possibility, apical ⁸⁶Rb efflux studies were performed. No difference was seen, however, between uninfected and infected monolayers (0.001 ± 0.0001 vs. 0.001 ± 0.0003 efflux rate constants for control and EPEC-infected monolayers; n = 8; P = 0.1). Second, the role of altered Na⁺ transport was explored in three ways. First, if inhibition of Na⁺ absorption were the cause of blunted I_{sc}, then removal of Na⁺ from the apical reservoir should increase stimulated I_{sc} back to the level seen in uninfected control monolayers. Removal of apical Na⁺ had no effect on I_{sc}. Second, apical Na⁺ absorption in the intestine occurs, in part, via the Na⁺/H⁺ exchanger 3 (NHE3). To inhibit NHE3, amiloride (100 μM) was added to the apical reservoir of monolayers before stimulation with secretagogues. The I_{sc} response in EPEC-infected monolayers was not altered by amiloride pretreatment. Amiloride also blocks Na⁺ channels; therefore, taken together, these data indicate that neither inhibition of Na⁺ absorption by NHE3 nor inhibition of Na⁺ channels accounts for the attenuation in I_{sc} associated with EPEC infection. Finally, that altered Na⁺ transport was not responsible for the observed diminution in I_{sc} was shown by performing unidirectional ²²Na⁺ fluxes (Table 1). There was minimal movement of Na⁺ in response to cAMP-mediated secretagogues as has been demonstrated previously (5). These data also confirm the integrity of the tight junctions, dispelling the possibility that enhanced paracellular permeability might account for some of the difference in I_{sc} between control and EPEC-infected monolayers.

EPEC infection alters HCO₃⁻ transport. Because investigations regarding the effect of EPEC on each of the major ions transported by intestinal epithelium revealed no significant differences, a role for HCO₃⁻ was considered. HCO₃⁻ transport by the basolateral Na⁺-HCO₃⁻ cotransporter is electrogenic; therefore, the impact of removal of basolateral HCO₃⁻ on forskolin-induced I_{sc} was examined in control and EPEC-infected monolayers. As shown in Fig. 5, removal of basolateral HCO₃⁻ decreases I_{sc} in EPEC-infected monolayers to a much greater extent than it does in controls. These findings suggest that a basolateral, HCO₃⁻-dependent

Table 1. Unidirectional and net Cl⁻ and Na⁺ fluxes across control and EPEC-infected T84 monolayers stimulated with forskolin

<table>
<thead>
<tr>
<th></th>
<th>J_{Cl} µeq h⁻¹ cm⁻²</th>
<th>J_{Na} µeq h⁻¹ cm⁻²</th>
<th>J_{Cl+Na} µeq h⁻¹ cm⁻²</th>
<th>Predicted I_{sc} µA cm⁻²</th>
<th>Measured I_{sc} µA cm⁻²</th>
<th>R, Ω cm⁻²</th>
<th>J_{Na+m} µeq h⁻¹ cm⁻²</th>
<th>J_{Na+s} µeq h⁻¹ cm⁻²</th>
<th>J_{Na} µeq h⁻¹ cm⁻²</th>
</tr>
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<tbody>
<tr>
<td>Control</td>
<td>1.17 ± 0.15</td>
<td>2.22 ± 0.12</td>
<td>-1.05 ± 0.03</td>
<td>27</td>
<td>45 ± 7.3</td>
<td>1.450 ± 159</td>
<td>0.39 ± 0.16</td>
<td>0.42 ± 0.24</td>
<td>-0.03 ± 0.01</td>
</tr>
<tr>
<td>EPEC infected</td>
<td>0.87 ± 0.28</td>
<td>1.98 ± 0.2</td>
<td>-1.11 ± 0.08</td>
<td>29</td>
<td>35 ± 3.4</td>
<td>1.305 ± 227</td>
<td>0.46 ± 0.24</td>
<td>0.41 ± 0.24</td>
<td>+0.05 ± 0.02</td>
</tr>
</tbody>
</table>

Values are means ± SE. EPEC, enteropathogenic E. coli. j_{Cl-m}, j_{Cl-s}, j_{Na-m}, and j_{Na-s} are mucosal-to-serosal, serosal-to-mucosal, and net Cl⁻ flux, respectively. j_{Na+m}, j_{Na+s}, and j_{Na} are mucosal-to-serosal, serosal-to-mucosal, and net Na⁺ flux, respectively. I_{sc}, short-circuit current; R, resistance.
were unidirectional Isc control and EPEC-infected monolayers. Initial studies suggest that the decrease in monolayers, however, some discrepancy persists that 25% net Cl₂ secretion is demonstrated to be stimulated by mucosal acidification (13). When Isc measurements are taken using our model, however, the original medium is removed and replaced with Ringer solution (pH 7.4) before challenge with secretagogues. To confirm that apical acidification is not the factor driving the altered Isc response, additional experiments were performed. First, acidified culture medium from infected T84 cells was removed, filtered, and added to new monolayers for 3 h. These new monolayers were then challenged with either forskolin or carbachol. There was no significant difference in the Isc response between control and supernatant-treated monolayers stimulated with forskolin (94 ± 4 vs. 86 ± 11 µA/cm² for control and infected monolayers; n = 8; P = 0.1) or carbachol (61 ± 4 vs. 56 ± 11 µA/cm² for control and EPEC-infected monolayers, respectively; n = 8, P = 0.5).

In other experiments, the mucosal medium of uninfected control monolayers was acidified to the pH seen in EPEC-infected monolayers (6.8), incubated for 3 h, and then challenged with the secretagogue. Again, no difference in the Isc response was observed (data not shown). These data indicate that mucosal acidification by EPEC is not responsible for the observed difference in net ion transport.

DISCUSSION

The pathophysiology underlying EPEC-associated diarrhea appears to be much more complex than initially realized. The originally described morphological lesion, which included microvillus degeneration, seemed initially to provide a logical explanation for the resultant diarrhea. That is, it was presumed that the loss of crucial microvillar brush-border enzymes resulted in malabsorption and osmotic diarrhea. Subsequent data resulting from several different avenues of investigation, however, suggest that this is not the case. For example, human volunteer studies have shown that the onset of diarrhea following the ingestion of EPEC occurs as early as 4 h (6), too short a time for the sequence of attachment, effacement, and malabsorption to occur. Also, EPEC adheres as microcolonies, not individual bacteria, thus infecting only a portion (~50%) of enterocytes (14). As such, a substantial number of unaffected absorptive cells remain. Furthermore, only those microvilli in close proximity to adherent EPEC are altered, leaving some normal-appearing microvilli

**Table 2.** Forskolin-stimulated unidirectional and net Cl⁻ fluxes in control and EPEC-infected T84 monolayers in the absence of HCO₃⁻

<table>
<thead>
<tr>
<th></th>
<th>J_{Cl,Δ}</th>
<th>J_{Cl,Δ}</th>
<th>J_{Cl,net}</th>
<th>Predicted Isc</th>
<th>Measured Isc</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>(µeq·h⁻¹·cm⁻²)</td>
<td>(µeq·h⁻¹·cm⁻²)</td>
<td>(µeq·h⁻¹·cm⁻²)</td>
<td>(µA/cm²)</td>
<td>(µA/cm²)</td>
</tr>
<tr>
<td>Control</td>
<td>0.61 ± 0.12</td>
<td>1.18 ± 0.2</td>
<td>-0.57 ± 0.07</td>
<td>15</td>
<td>15.5 ± 0.85</td>
</tr>
<tr>
<td>EPEC infected</td>
<td>0.59 ± 0.16</td>
<td>1.15 ± 0.24</td>
<td>-0.56 ± 0.05</td>
<td>15</td>
<td>11 ± 1.18</td>
</tr>
</tbody>
</table>

Values are means ± SE.
on the infected enterocyte. Last, although EPEC infection undoubtedly accelerates the normal process of vesicular shedding of microvillar digestive enzymes (2), there is evidence to suggest that the enterocyte may respond by a compensatory increase in the synthesis of such enzymes (7). These data, in addition to more recent studies that examine in detail the impact of EPEC infection on intestinal epithelial cell function, suggest that the pathophysiology of EPEC-associated diarrhea is quite complex and cannot be simply attributed to loss of brush-border enzymes.

The elucidation of the pathophysiology underlying EPEC-associated diarrhea is further complicated by the finding that EPEC does not induce active ion secretion. In an early study by Moon et al. (20), it was reported that infection of ligated ileal loops by EPEC in both pigs and rabbits did not induce fluid accumulation despite the presence of the attaching and effacing lesion. Interestingly, infection was found more frequently and was characterized as being more extensive in the large vs. the small intestine. Colonic loop experiments were not performed in this study.

More recently, attention has shifted to examining the effects of EPEC specifically on its host target cell, the intestinal epithelial cell. These studies have yielded interesting information. In contrast to the usual paradigm of bacteria-induced Cl− secretion, EPEC has been shown herein and by others (23) to not activate this process. In fact, net ion secretion stimulated by classic secretagogues is blunted in EPEC-infected monolayers. The results presented herein suggest that such attenuation is a global phenomenon in that both Ca2+- and cAMP-stimulated Isc values are decreased. Philpott et al. (23), however, demonstrated attenuated Isc only when stimulated with forskolin, not carbachol. This scenario is reminiscent of what occurs when the actin cytoskeleton is stabilized with agents such as phalloidin (19). In this case, diminished Isc in response to forskolin is attributable to inhibition of the basolateral Na+-K+–2Cl− cotransporter. It was, therefore, hypothesized that the effect of EPEC on forskolin-stimulated Isc, which was presumed to reflect diminished Cl− secretion, may be linked to this same mechanism. These investigators, however, did not examine the effect of EPEC on Cl− secretion specifically but only examined Isc, a measurement of net ion transport. Furthermore, EPEC has not been shown to alter basal activity, a component demonstrated to regulate Na+-K+–2Cl− cotransporter activity.

The data presented herein show that the diminution in stimulated Isc caused by infection with EPEC is, surprisingly, not due to diminished Cl− secretion. This was demonstrated by showing that each specific transporter participating in apical Cl− secretion, including apical Cl− channels and the basolateral Na+-K+–2Cl− cotransporter, was not altered by EPEC. In addition, Cl− flux studies performed on Ussing chambers confirmed that net Cl− secretion was identical in control and infected monolayers. It should be noted, however, that a recent publication reported that EPEC infection of Caco-2 monolayers induced a rapid but transient increase in Isc, a portion (~25%) of which was Cl− dependent (4). The peak change in Isc was seen at 10-min postinfection; however, a model of synchronized infection was employed whereby bacteria were centrifuged onto the cell surface. Obviously, the differences in the model employed, both cell line and method of infection, could account for the variability in results.

Our studies also suggest that altered Na+ absorption does not account for the diminished Isc seen in EPEC-infected monolayers. Neither removal of Na+ from the apical buffer nor treatment with amiloride, which inhibits NHE3 as well as Na+ channels, altered the attenuated Isc response. In addition, Na+ flux studies showed no difference in Na+ transport.

It is becoming increasingly apparent that CFTR serves not only as a conductor for the active secretion of Cl− but also for HCO3− (3, 12, 13). The role of CFTR in HCO3− secretion is, however, more complex. Much like Cl− secretion, the secretion of HCO3− requires the orchestration of several transporters, including an anion channel, apical Cl−/HCO3− exchanger, and basolateral uptake by the Na+-HCO3− cotransporter. Intracellular HCO3− may also be generated through the action of carbonic anhydrase. The effect of cAMP and Ca2+ stimulation on these various components involved in HCO3− secretion is just beginning to be investigated.

The contribution of HCO3− to secretagogue-stimulated Isc in T84 cells has not been addressed. Our studies suggest, contrary to popular dogma, that HCO3− may be responsible for up to 40% of forskolin-stimulated Isc, the difference between calculated and measured Isc shown herein. In reviewing the data published as a part of the initial characterization of the transport properties of T84 cells (5), the measured Isc was 20% greater than that predicted from the net secretion of Cl−. This unexplained Isc may be attributable to HCO3− secretion. Our Cl− flux studies performed in the absence of HCO3− showed that both predicted and measured Isc dropped significantly. This does not necessarily imply that HCO3− secretion directly accounts for nearly one-half of the generated Isc. Instead, it has been proposed that the activities of CFTR and the apical Cl−/HCO3− exchanger are linked (3). One offered explanation of this interaction is that the Cl−/HCO3− exchanger serves to recycle Cl− through the apical membrane (3). If Cl−/HCO3− activity is interrupted, either by direct inhibition or removal of HCO3−, then there may be a decrease in net Cl− secretion as we observed in our flux studies. Under these conditions, measured and predicted Isc corresponded perfectly.

Regarding the effect of EPEC infection on stimulated net ion transport, our studies clearly demonstrate that diminished Cl− secretion does not account for the observed difference in Isc. Examination of the influence of EPEC on the transport of other major ions, namely Na+ and K+, failed to reveal any change. That diminished transepithelial resistance was not responsible for the measured changes in active ion transport was shown in two ways. First, alterations in secretagogue-stimulated Isc were demonstrated before changes in

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permeability, as shown in Fig. 2. Second, if altered paracellular permeability were responsible, the flux rate of Na\(^+\) would have increased. Furthermore, a mutant strain of EPEC known to not decrease epithelial resistance still induces alterations in I\(_{sc}\).

How, then, might the EPEC-induced diminution in I\(_{sc}\) be explained? In that the degree of decrease in net ion transport is quite substantial and alterations in the transport of none of the major ions, including Cl\(^-\), Na\(^+\), and K\(^+\), appear to account for this change, HCO\(_3\)\(^-\) is a likely candidate. When HCO\(_3\) is removed from uninfected control monolayers, secretagogue-stimulated I\(_{sc}\) decreases significantly, ranging from 20 to 40%. In this case, I\(_{sc}\) approximates that seen in EPEC-infected monolayers. We, therefore, propose that EPEC is altering the transport of HCO\(_3\)\(^-\). Whether this change is being effected at the apical or basolateral membrane cannot be distinguished at this point. Also, the complex interaction that appears to exist between CFTR and the Cl\(^-\)/HCO\(_3\) exchanger makes the regulation of HCO\(_3\)\(^-\) secretion difficult to unravel. Clarke and Harline (3) suggest that, in the duodenal epithelium, the relative contribution of CFTR as a conductor of HCO\(_3\) and as a “facilitator” of apical Cl\(^-\)/HCO\(_3\) exchange likely changes in response to cues from the luminal environment. In addition, the activities of the basolateral Na\(^+\)-HCO\(_3\) cotransporter and intracellular carbonic anhydrase must be considered. To reconcile the specific mechanisms by which EPEC interferes with HCO\(_3\) transport, many additional studies will be required. EPEC-infected intestinal epithelial cells should, however, provide an interesting model for deciphering the complex pathways that regulate HCO\(_3\)\(^-\) secretion.

Although these studies enhance our understanding of the effects of EPEC infection on active ion transport, they do not fit the usual paradigm for diarrhea that occurs in response to infection by enteric pathogens. It has been shown that with time EPEC increases the permeability of tight junctions by inducing the phosphorylation of myosin light chain (27). This event may contribute to the production of diarrhea, but it is likely that other factors are involved as well.

In vivo, EPEC infection induces the recruitment of inflammatory cells, primarily neutrophils, not only into the lamina propria but also across intact crypts (20). Activated neutrophils produce 5\(^\prime\)-AMP, which is converted by epithelial cell 5\(^\prime\)-ectonucleotidase to adenosine, a potent secretagogue (17). In our in vitro model, EPEC infection also attenuates I\(_{sc}\) in response to adenosine (unpublished observation). In vivo, however, EPEC attaches primarily to surface epithelial cells and less often to crypt cells, causing severe villous atrophy and crypt hyperplasia (22). Neutrophils tend to accumulate in intestinal crypts where it would be possible for uninfected crypt cells in the in vivo situation to demonstrate a full secretory response to neutrophil-derived adenosine.

In summary, the studies presented herein confirm that EPEC infection of intestinal epithelial cells diminishes net ion transport, as measured by I\(_{sc}\) in response to classic secretagogues. Unexpectedly, our results clearly show that this decrease in net ion transport does not represent diminished Cl\(^-\) secretion as one might predict. Instead, it appears that EPEC alters HCO\(_3\)\(^-\) secretion and possibly perturbs other HCO\(_3\)-dependent transport processes. Additional studies are needed to further define these transport changes.

We are grateful to our colleagues, Drs. RamaSawmy and Rao, for insightful suggestions regarding these studies.

This work was supported by National Institute of Diabetes and Digestive and Kidney Diseases Grant DK-50694 and the Department of Veterans Affairs (Merit Award) to G. Hecht.

This work was presented in preliminary form at the annual meeting of the American Gastroenterological Association held May 16–20, 1998, in New Orleans, LA.

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Received 6 July 1998; accepted in final form 3 December 1998.

REFERENCES


5. Dharmathaphorn, K. K., G. G. Mandel, H. Masui, and J. 787). 840 South Wood St., CSB Rm. 704, Chicago, IL 60612 (E-mail: gahecht@uiuc.edu).

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