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NOTES

Enteropathogenic *Escherichia coli* Markedly Decreases the Resting Membrane Potential of Caco-2 and HeLa Human Epithelial Cells

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It is presumed, but not proven, that enteropathogenic *Escherichia coli* (EPEC) causes secretory diarrhea by altering ion transport in enterocytes. In this study we used the whole-cell, current clamp variant of the patch clamp technique to demonstrate that EPEC infection of HeLa and Caco-2 human epithelial cells reduces cell resting membrane potential. The observed reduction of resting membrane potential in HeLa cells results from EPEC-mediated signal transduction to the host cell but is not dependent upon EPEC-mediated elevation of levels of intracellular free calcium. These findings indicate that EPEC can directly alter the relative distribution of ions across epithelial host cell membranes. This may be relevant to the etiology of diarrhea caused by EPEC infection.

Enteropathogenic *Escherichia coli* (EPEC) causes a diarrheal disease that is a leading cause of infant death in developing nations (26). However, the mechanisms by which EPEC causes diarrhea are unknown. Clinical findings indicate that EPEC-mediated diarrhea contains high levels of sodium and is therefore classified as a secretory-type diarrhea (6, 16, 23). Secretory diarrhea arises from either the secretion of ions into the gut or the inability of the gut to absorb ions from the luminal contents (4). Increased ion concentrations in the gut will cause an increase in water content in the stool and, possibly, a diarrheal disease. Since EPEC does not produce toxins (25), it is presumed that the direct interaction of EPEC with intestinal epithelial cells either alters the ability of these cells to transport ions from the gut lumen or triggers ion secretion by these cells.

EPEC-infected human intestinal cells exhibit several distinctive features. Histopathological findings indicate that EPEC adheres to intestinal epithelial cells and forms microcolonies on the apical cell surface (39). The membranes of enterocytes and bound EPEC closely associate in a manner termed intimate adherence. Intimately adherent EPEC rests upon a cup-like pedestal, and microvilli are effaced, resulting in formation of an attaching and effacing lesion.

In vitro studies support a multistage model for the interactions of EPEC with epithelial cells that result in the observed histopathological changes seen in vivo (21). The first stage involves the initial adherence of EPEC to epithelial cells as microcolonies via the bundle-forming pilus (13). The second stage occurs when bound EPEC triggers host signal transduction pathways to initiate cytoskeletal rearrangements (30). The bacterial factors required for host cell signaling include secretion of EaeB, also referred to as EspB, and other proteins via a type III export system encoded by the *sep* genes (10, 19, 22). EPEC signaling induces tyrosine phosphorylation of several host proteins, including a predominant 90-kDa membrane protein (Hp 90). These phosphorylated proteins cluster directly beneath the adherent EPEC (29). EPEC signaling also mediates release of inositol phosphates (IP_3) and calcium in the host cell cytoplasm (7, 11). The third stage of interaction involves EPEC-directed alterations of the host cell cytoskeleton typified by filamentous actin focused beneath EPEC, intimate adherence, and pedestal formation (9, 24). An EPEC outer membrane protein, intimin, encoded by *eaeA*, mediates these interactions by binding to Hp 90 and focusing cytoskeletal rearrangements beneath the adherent bacteria (20, 32).

Findings from these in vitro studies have suggested possible operating mechanisms that underlie EPEC-mediated diarrheal disease. These include altered enterocyte ion transport resulting from host cell signal cascades triggered by IP_3 fluxes or by elevated levels of intracellular free calcium (3, 7). However, the necessary prerequisite finding, that EPEC does actually alter ion distribution across cultured epithelial cell membranes, has not been demonstrated. Therefore, to determine if EPEC does alter the distribution of ions across individual host cells, we measured the effect of EPEC on the resting membrane potential (RMP) of infected HeLa and Caco-2 epithelial cells. To determine the stage in EPEC epithelial cell interactions at which alterations in RMP occur, we also examined HeLa cells infected with EPEC mutants that are deficient for signal transduction (*eaeB* and class four mutants [cfm]) or intimate adherence (*eaeA*). The RMP was determined via the whole-cell, current clamp variant of the patch clamp technique. This measurement represents the voltage at which there is no net movement of positive or negative ions across the cell membrane (33). Therefore, the RMP is the result of, and directly reflects, the collective action of epithelial cell ion transport,

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TABLE 1. EPEC strains used in this study

Strain	Adherence in microcolonies	IP_3 release	$[Ca^{2+}]_i$ increase	HP 90 phosphorylation	Cytoskeletal rearrangement	Decrease in level of transepithelial resistance	Decrease in RMP[a]
E2348/69, parental EPEC	+	+	+	+	+	+	+
10-5-1(1) *eaeA*::Tn*phoA*	+	+	−	+	±[b]	−	+
14-2-1(1) (cfm) *sep2*::Tn*PhoA*	+	−	ND[c]	−	−	−	−
UMD864 Δ*eaeB*	+	−	ND	−	−	ND	−

[a] Determined in this study.
[b] The cytoskeletal rearrangements were limited and were not focused beneath the adherent bacteria.
[c] ND, not determined.

whether by means of ion channels or pumps. If EPEC affects the movement of ions across the epithelial cell membrane, changes in the RMP of cultured epithelial cells will occur.

Strains and culture conditions used. The EPEC strains used in this study are as shown in Table 1. Human epithelial-like HeLa cells (ATCC CCL2) and human enterocyte-like Caco-2 cells (ATCC HTB37) were grown on 12-mm-diameter coverslips as described previously (10–12). HeLa cells were used at 50 to 70% confluency 2 days after seeding, and Caco-2 cells were used 4 to 6 days after seeding and grew in islets of 100 to 200 cells. Epithelial cells were either left untreated or infected with preinduced EPEC. For some experiments, HeLa cells were treated with drugs or incubated in the presence of both preinduced EPEC and drugs, as indicated in Table 2. Infection of HeLa or Caco-2 cells was performed as follows. EPEC strains were preinduced for microcolony formation (31). Preinduced EPEC were diluted 1:2 with fresh minimal essential medium (MEM) and added to epithelial cells. After 30 min, nonadherent bacteria were removed by washing the monolayers with MEM. Preinduced EPEC rapidly adhere to epithelial cells as microcolonies and transduce signals or cause the accumulation of phosphotyrosine-containing proteins and filamentous-actin under the adherent EPEC (data not shown and reference 31). These EPEC-mediated signaling events were observed as early as 5 min following the infection procedure. Infected epithelial cells were used for patch clamp analysis 20 min to 2 h following infection. This study also used several EPEC mutant strains with defects in their interaction with cultured epithelial cells. EPEC signal transduction mutants contain lesions either within the *sep* genes, termed cfm, or within *eaeB*. These strains do not trigger Hp 90 phosphorylation or an elevation of IP_3 (10, 11, 29). Intimate adherence mutants have a lesion within *eaeA*, and although they trigger Hp 90 phosphorylation and an elevation of levels of IP_3, they are unable to focus Hp 90 and cytoskeletal rearrangements beneath the adherent bacteria (29). These signal transduction and intimate adherence mutants formed microcolonies following preinduction, as did the parental EPEC, and exhibited the phenotypes expected for these mutant strains (data not shown and references 10, 11, and 29).

Patch clamp recording methods. Patch clamp recordings from HeLa and Caco-2 cells were obtained by methods described previously (18). We recorded RMPs with the whole-cell variant of the patch clamp technique, using standard extracellular saline (SES) as a cell bathing solution and standard internal saline to fill patch electrodes (15). The Ca^{2+}-free extracellular solution had the same composition as SES except for the omission of $CaCl_2$ and the addition of 3 mM EGTA [ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid]. Recordings were obtained at room temperature (22 to 24°C) and completed within 1 h of cell removal from the incubator. In the case of EPEC-infected HeLa or Caco-2 cells, recordings were made with cells displaying at least one attached microcolony. Caco-2 cells or EPEC-infected Caco-2 cells selected for analysis were from within islets consisting of 100 to 200 cells (12). We routinely evaluated the viability of infected epithelial cells by trypan blue exclusion. No detectable drop in the level of viability of HeLa or Caco-2 cells infected

TABLE 2. HeLa cell incubation protocols used in this study

Experimental treatment	First incubation (37°C)		Second incubation (37°C)		Patch clamp recording (22–24°C)	
	Medium	Duration (min)	Medium	Duration (min)	Bath saline solution	Duration (min)[a]
No treatment	MEM	60			SES	60
EPEC	MEM and EPEC	30			SES	60
EPEC mutants	MEM and mutants	30			SES	60
EPEC supernatant	Filtered supernatant of EPEC-containing medium	30			SES	60
Ouabain	MEM and ouabain	60			SES and ouabain	60
Ouabain plus EPEC	MEM and ouabain	30	MEM, ouabain, and EPEC	30	SES and ouabain	60
Thapsigargin	MEM and thapsigargin	60			SES and thapsigargin	60
Thapsigargin plus EPEC	MEM and thapsigargin	30	MEM, thapsigargin, and EPEC	30	SES and thapsigargin	60
BAPTA-AM	MEM and BAPTA-AM	60			SES and BAPTA-AM	60
BAPTA-AM plus EPEC	MEM and BAPTA-AM	30	MEM, BAPTA-AM, and EPEC	30	SES and BAPTA-AM	60
0Ca^{2+}-BAPTA-AM	Ca^{2+}-free MEM and BAPTA-AM	60			Ca^{2+}-free ES and BAPTA-AM	60
0Ca^{2+}-BAPTA-AM plus EPEC	Ca^{2+}-free MEM and BAPTA-AM	30	Ca^{2+}-free MEM, BAPTA-AM, and EPEC	30	Ca^{2+}-free ES and BAPTA-AM	60

[a] All patch clamp recordings were completed within 60 min of cell removal from the incubator.

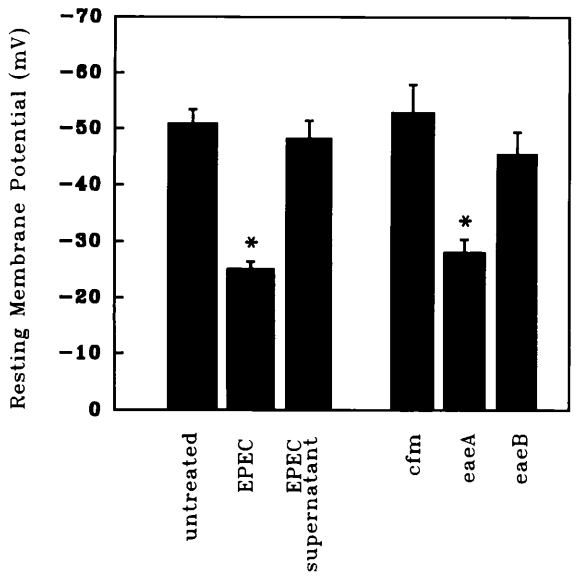

FIG. 1. Effect of incubation with wild-type EPEC and EPEC mutant strains on the RMP of HeLa cells in SES, as recorded by whole-cell patch clamp methods. Patch electrodes were filled with standard internal saline. Mean RMPs were obtained from HeLa cells incubated with normal culture medium (n = 23 cells), EPEC-containing medium (n = 13 cells), cfm-containing medium (n = 13 cells), *eaeA*-containing medium (n = 11 cells), or *eaeB*-containing medium (n = 14 cells). A final group of 10 cells was incubated in the filtered supernatant from wild-type EPEC-containing culture medium. Only mean RMPs obtained after infection with either wild-type EPEC or *eaeA* strains were significantly lower than that of the control ($P < 0.001$, ANOVA, asterisks). Data are expressed as means ± standard errors of the means.

with EPEC strains was observed under the experimental conditions used in this study.

EPEC depolarizes HeLa and Caco-2 cell membranes. HeLa cells which exhibited at least one attached wild-type EPEC microcolony had a significantly lower mean RMP than was observed in cells incubated in MEM alone (Fig. 1). The mean RMP for untreated HeLa cells was −51 ± 2.4 mV (n = 23 cells), and the mean RMP for EPEC-treated cells was −25 ± 1.2 mV (n = 13 cells, $P < 0.001$, by analysis of variance [ANOVA]). Incubation of HeLa cells with the supernatant from preinduced EPEC cultures failed to elicit a drop in RMP relative to control values. The mean RMP for 10 HeLa cells treated with EPEC supernatant was −48 ± 3.0 mV (Fig. 1). This indicates that the EPEC-mediated drop in RMP was not due to the alteration of medium composition by bacterial growth or the elaboration of bacterial components into the medium.

We also examined the effect of EPEC on the RMP of the enterocyte-like Caco-2 cell line. As with HeLa cells, EPEC reduced the RMP of Caco-2 cells to half of its normal value. The mean RMP for untreated Caco-2 cells was −38.4 ± 2.0 mV (n = 12 cells), while the mean RMP for EPEC-treated cells was −19.6 ± 1.5 mV (n = 11 cells, $P < 0.001$, ANOVA).

Signal transduction by EPEC causes the observed depolarization of epithelial cell membranes. In order to determine what stage in the interaction of EPEC with epithelial cells leads to the observed reduction of RMP, we used HeLa cells infected with EPEC mutants deficient for intimate adherence (*eaeA*) or signal transduction (cfm or *eaeB*). HeLa epithelial cells were selected, since they have been extensively characterized in terms of EPEC-mediated signal transduction and cytoskeletal rearrangements (9–11, 22, 31) and in terms of their electrophysiological properties (34–36). The *eaeA* mutant depolarized HeLa cells to the same degree as wild-type EPEC (mean value, −28 ± 2.2 mV, n = 11 cells, $P > 0.05$ with wild-type data, ANOVA). In contrast, RMPs recorded from HeLa cells infected with either the *eaeB* (mean value −45 ± 4 mV, n = 14) or cfm mutants (mean value, −52 ± 6 mV, n = 13 cells) did not differ significantly from control values ($P > 0.05$, ANOVA) (Fig. 1). Therefore, the observed reduction in RMP requires the EPEC-mediated signaling of HeLa cells by EaeB and possibly other secreted proteins.

We treated HeLa cells with 0.1 mM ouabain to demonstrate that specific inhibition of an ion transport protein could lead to a reduction in the RMP comparable to that mediated by EPEC. Ouabain is a member of a family of glycosides used in cardiac therapy which binds to and inactivates the Na^+/K^+ ATPase of eucaryotic cells (17). The Na^+/K^+ ATPase plays an essential role in maintaining transmembrane gradients of potassium and sodium ions in animal cells (38). Incubation of HeLa cells with ouabain reduced the HeLa cell RMP to −26 ± 1.8 mV (n = 16 cells), a value similar to that produced by EPEC infection ($P > 0.05$, ANOVA). When HeLa cells were incubated in the presence of both ouabain and EPEC, cell RMP dropped by an extent slightly greater than was seen with either treatment alone. The mean RMP was −20 ± 1.7 mV (n = 12 cells, $P < 0.05$ with ouabain only or EPEC only data, ANOVA). The effects of these two treatments on RMP were therefore only weakly additive. Nevertheless, it is unlikely that residual pump activity persisted at the concentration of ouabain employed (1), suggesting that the EPEC-mediated reduction in RMP is not simply due to the inhibition of the Na^+/K^+ ATPase.

Calcium is not a mediator of HeLa cell depolarization by EPEC. In order to explore possible mechanisms for EPEC-mediated HeLa cell depolarization, we investigated the possible role of the EPEC-mediated rise in the level of free intracellular calcium (2, 3, 7). EPEC-mediated elevations in levels of free intracellular calcium were blocked by loading HeLa cells with the membrane-permeable calcium chelator BAPTA-AM [1,2-bis(2-aminophenoxy)ethane-*N*,*N*,*N*′-tetraacetic acid acetoxy methylester, 100 μM] prior to infection with EPEC. The loading of HeLa cells with BAPTA-AM resulted in a small reduction in the HeLa cell RMP (see Fig. 3) when measured in medium containing normal levels of Ca^{2+} or in calcium-free media supplemented with 3 mM EGTA ($0Ca^{2+}$) (Table 2). EPEC infection of BAPTA-AM-loaded HeLa cells in either Ca^{2+} or Ca^{2+}-free medium resulted in significant further depolarizations ($P < 0.05$, ANOVA) (see Fig. 3). To confirm that BAPTA-AM effectively buffered elevations in intracellular free calcium, we measured calcium levels in BAPTA-AM-treated HeLa cells by fluorescence calcium imaging (Fig. 2). HeLa cells were loaded with the cell-permeant calcium indicator fura 2-AM (Molecular Probes, Inc., Eugene, Oreg.) by a 90-min incubation in 5 μM fura 2 solution at 22 to 24°C. Fura 2-AM-loaded cells were incubated at 37°C during monitoring of free intracellular calcium, $[Ca^{2+}]_i$, which was determined by the ratio of fluorescence signals at 334- and 380-nm wavelengths (14). These cells were then loaded with BAPTA-AM, and the effectiveness of this reagent was assessed. As shown in Fig. 2, BAPTA-AM effectively buffered the rise in intracellular free calcium triggered in HeLa cells by the application of histamine (27). Therefore, chelation of intracellular or both intracellular and extracellular calcium did not block the depolarizing influence of EPEC on HeLa cell membranes.

The role of calcium was also examined with the drug thapsigargin. Treatment of HeLa cells with thapsigargin (2 μM) depletes the intracellular Ca^{2+} stores of these cells by inhibit-

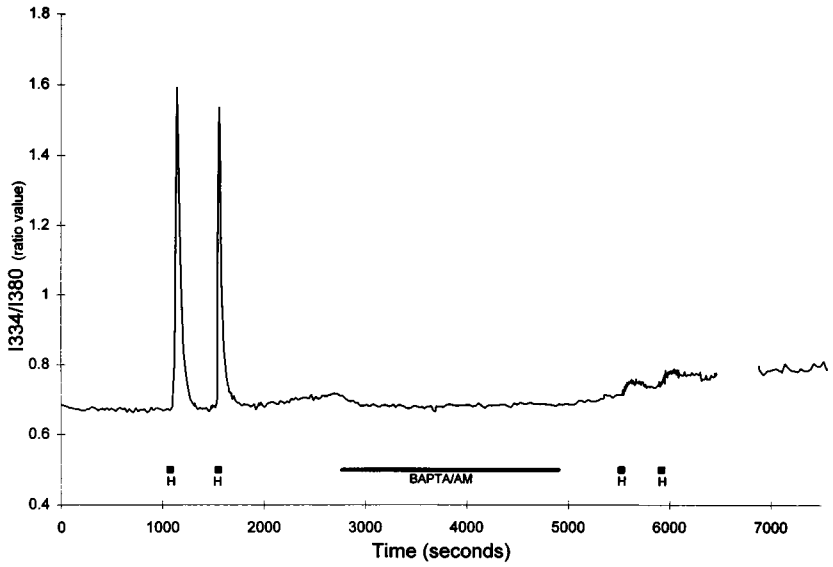

FIG. 2. Preincubation with BAPTA-AM-suppressed histamine-induced fluxes in $[Ca^{2+}]_i$ in HeLa cells. $[Ca^{2+}]_i$ was monitored as the ratio of fluorescent signals detected at 334- and 380-nm wavelengths (I334/I380). Prior to BAPTA-AM loading, two 50-s applications of 1 mM histamine (H) to the cells each resulted in rapid and reversible increases in the fluorescence ratio, indicating increases in $[Ca^{2+}]_i$. Following loading of these cells with BAPTA-AM (horizontal bar), the application of histamine evoked negligible changes in $[Ca^{2+}]_i$. The plotted fluorescence ratio signal represents the mean ratio for 20 cells in the microscope field.

ing the intracellular Ca^{2+} pumps and preventing the sequestration of Ca^{2+} to intracellular stores (27). HeLa cells treated with thapsigargin in medium containing a normal amount of calcium exhibited a slight drop in RMP (Fig. 3). Incubation of thapsigargin-treated HeLa cells with EPEC resulted in a significantly greater reduction in RMP compared with HeLa cells treated with thapsigargin alone (Fig. 3). This depolarization was not significantly different from that seen in cells exposed to EPEC alone, indicating that thapsigargin treatment did not prevent EPEC-mediated depolarization in HeLa cells.

Finally, *eaeA* mutants do not cause an elevation in levels of intracellular free calcium, nor do they cause the calcium-dependent drop in the level of transepithelial resistance observed in polarized epithelial monolayers (7, 37). As stated previously, the *eaeA* mutant did cause a drop in RMP in infected HeLa cells. Taken collectively, these results indicate that the drop in RMP observed in EPEC-treated cells is not mediated by calcium-dependent signal transduction pathways or by calcium-mediated cytotoxicity (reviewed in reference 28).

This study provides the first direct evidence that EPEC infection alters the distribution of ions across the cell membrane of both HeLa and Caco-2 human epithelial cells, reducing the RMP to about half its normal value. In principle, EPEC-mediated depolarization could result directly from an influx of positive ions or from an efflux of negative ions across the epithelial cell membrane. Alternatively, EPEC may act indirectly by inhibiting metabolically driven pumps which maintain the large transmembrane concentration gradients for K^+, Na^+, and Cl^-, upon which the resting potential ultimately depends. Any marked fall in resting potential greatly reduces the electrochemical gradient available for Na^+ entry into the epithelial cell. In the context of an intact gut epithelium, this change would lead to accumulation of Na^+ and water in the gut lumen. This would result in the formation of a profuse, sodium-rich diarrhea, as is seen clinically in EPEC infections. The depolarizing effect of EPEC infection was similar in magnitude to that caused by exposure of HeLa cells to the Na^+/K^+ ATPase-blocking drug ouabain. It is of interest that diarrhea is a common side effect of clinically used ouabain analogs and may indeed be the only gastrointestinal symptom of cardiac glycoside toxicity (17).

Previous studies exploring the effects of EPEC on cultured epithelial cell electrophysiology measured transcellular resistance across polarized monolayers (5, 37). The reported reduction in the level of polarized monolayer resistance differs from the decrease in HeLa and Caco-2 cell RMP in two respects. First, *eaeA* mutants failed to cause a fall in the level of transepithelial resistance yet were identical to wild-type EPEC in their ability to depolarize HeLa and Caco-2 cells. Second, the fall in the level of transepithelial resistance was seen only after prolonged incubation with EPEC organisms, while EPEC-mediated depolarization occurred as early as 30 min after bacterial attachment to HeLa cell membranes. These differences suggest that distinct signal transduction mechanisms may underlie the EPEC-mediated fall in the level of transepithelial resistance and in membrane potential. The drop in the level of transepithelial resistance requires the EPEC-mediated rise in the level of intracellular free calcium (37) and probably reflects activation of Ca^{2+}-dependent membrane conductances or calcium-mediated cytotoxicity (2). These processes are apparently not essential for EPEC-mediated cell depolarization. Our conclusion is supported by the finding that chelation of intracellular calcium with BAPTA-AM failed to protect HeLa cells against EPEC-mediated depolarization.

Our studies with EPEC mutants indicate that neither the initial attachment nor the intimate adherence of bacteria to host cells was itself sufficient to trigger EPEC-mediated depolarization. Similarly, the fall in resting potential could not be attributed to the elaboration of EPEC products into culture medium or the depletion of culture medium by the microorganisms. Rather, mutants which do not trigger signal transduction, including Hp 90 phosphorylation and IP_3 fluxes, were selectively unable to depolarize HeLa cells. Both of these signal transduction pathways could, in principle, influence the transport of ions across cell membranes.

Patch clamp techniques have been used previously to exam-

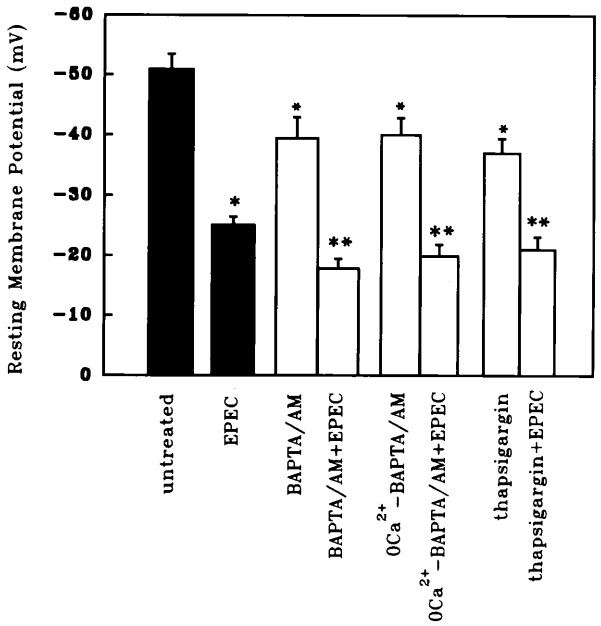

FIG. 3. EPEC-induced depolarization of HeLa cells was not blocked by pretreatment of HeLa cells with BAPTA-AM (100 μM), a chelator of intracellular Ca^{2+}, or with thapsigargin (2 μM), an inhibitor of intracellular Ca^{2+} pumps. Incubation in BAPTA-AM-containing medium with a normal level of Ca^{2+} ($n = 9$ cells) or without Ca^{2+} ($n = 6$ cells) itself significantly depolarized HeLa cells, compared with values for untreated cells ($n = 23$ cells, $P < 0.001$, ANOVA, single asterisks). However, coincubation of HeLa cells with media containing both wild-type EPEC and either BAPTA-AM with Ca^{2+} ($n = 8$ cells) or BAPTA-AM without Ca^{2+} ($n = 8$ cells) resulted in significantly greater depolarizations than were seen in cells treated with BAPTA-AM alone ($P < 0.05$, ANOVA, double asterisks). Similarly, incubation with thapsigargin-containing medium ($n = 13$ cells) also depolarized HeLa cells ($P < 0.001$, ANOVA, single asterisk). Coincubation of HeLa cells with medium containing both thapsigargin and EPEC ($n = 11$ cells) induced greater depolarization than was observed in cells treated with thapsigargin alone ($P < 0.05$, ANOVA, double asterisks). The mean depolarizations seen after coincubation of EPEC and BAPTA-AM in normal or calcium-free medium were significantly larger than that seen following treatment with EPEC alone ($P < 0.05$, ANOVA). Coincubation with thapsigargin and EPEC resulted in a depolarization not significantly different from that seen after exposure to EPEC alone.

ine the pathogenic actions of bacterial toxins (8). However, this study is the first example of applying this method to examine the direct interaction of live pathogenic bacteria with individual host cells. By utilizing this technique, we have shown that EPEC mediates a drop in epithelial cell RMP, thereby identifying a potential pathogenic mechanism which may contribute to diarrhea.

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REFERENCES

1. **Ash, F. J., R. M. Fineman, T. Kalko, M. Morgan, and B. Wire.** 1984. Amplification of sodium- and potassium-activated adenosine triphosphatase in HeLa cells by ouabain step selection. J. Cell Biol. **99:**971–983.
2. **Baldwin, T. J., M. B. Lee-Delaunay, S. Knutton, and P. H. Williams.** 1993. Calcium-calmodulin dependence of actin accretion and lethality in cultured HEp-2 cells infected with enteropathogenic *Escherichia coli*. Infect. Immun. **61:**760–763.
3. **Baldwin, T. J., W. Ward, A. Aitken, S. Knutton, and P. H. Williams.** 1991. Elevation of intracellular free calcium levels in HEp-2 cells infected with enteropathogenic *Escherichia coli*. Infect. Immun. **59:**1599–1604.
4. **Barrett, K. E., and K. Dharmsathaphorn.** 1991. Secretion and absorption: small intestine and colon, p. 265–294. *In* T. Yamada (ed.), Textbook of gastroenterology, vol. 1. J. B. Lippincott, Philadelphia, Pa.
5. **Canil, C., I. Rosenshine, S. Ruschkowski, M. S. Donnenberg, J. B. Kaper, and B. B. Finlay.** 1993. Enteropathogenic *Escherichia coli* decreases the transepithelial electrical resistance of polarized epithelial monolayers. Infect. Immun. **61:**2755–2762.
6. **Donnenberg, M. S., C. O. Tacket, S. P. James, G. Losonsky, J. P. Nataro, S. S. Wasserman, J. B. Kaper, and M. M. Levine.** 1993. Role of *eaeA* gene in experimental enteropathogenic *Escherichia coli* infection. J. Clin. Invest. **92:**1412–1417.
7. **Dytoc, M., L. Fedorko, and P. M. Sherman.** 1994. Signal transduction in human epithelial cells infected with attaching and effacing *Escherichia coli in vitro*. Gastroenterology **106:**1150–1161.
8. **Eriksen, S., S. Olsnes, K. Sandvig, and O. Sand.** 1994. Diphtheria toxin at low pH depolarizes the membrane, increases the membrane conductance and induces a new type of ion channel in Vero cells. EMBO J. **13:**4433–4439.
9. **Finlay, B. B., I. Rosenshine, M. S. Donnenberg, and J. B. Kaper.** 1992. Cytoskeletal composition of attaching and effacing lesions associated with enteropathogenic *Escherichia coli* adherence to HeLa cells. Infect. Immun. **60:**2541–2543.
10. **Foubister, V., I. Rosenshine, M. S. Donnenberg, and B. B. Finlay.** 1994. The *eaeB* gene of enteropathogenic *Escherichia coli* is necessary for signal transduction in epithelial cells. Infect. Immun. **62:**3038–3040.
11. **Foubister, V., I. Rosenshine, and B. B. Finlay.** 1994. A diarrheal pathogen, EPEC, triggers a flux of inositol phosphates in infected epithelial cells. J. Exp. Med. **179:**993–998.
12. **Gaillard, J.-L., and B. B. Finlay.** 1996. Effect of cell polarization and differentiation on entry of *Listeria monocytogenes* into the enterocyte-like Caco-2 cell line. Infect. Immun. **64:**1299–1308.
13. **Girón, J. A., A. S. Y. Ho, and G. K. Schoolnik.** 1991. An inducible bundle-forming pilus of enteropathogenic *Escherichia coli*. Science **254:**710–713.
14. **Grynkiewicz, G., M. Poenie, and R. Y. Tsien.** 1985. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. J. Biol. Chem. **260:** 3440–3450.
15. **Hamill, O. P., A. Marty, E. Neher, B. Sakmann, and F. J. Sigworth.** 1981. Improved patch-clamp techniques for high-resolution current recording from cells and cell-free membrane patches. Pflueg. Arch. **391:**85–100.
16. **Hill, S. M., A. D. Phillips, and J. A. Walker-Smith.** 1991. Enteropathogenic *Escherichia coli* and life threatening chronic diarrhoea. Gut **32:**154–158.
17. **Hoffman, B. F., and J. T. Bigger.** 1990. Digitalis and allied cardiac glycosides, p. 814–839. *In* A. G. Gilman, T. W. Rall, A. S. Nies, and P. Taylor (ed.), The pharmacological basis of therapeutics, 8th ed. Pergamon Press, New York.
18. **Hong, Y., E. Puil, and D. A. Mathers.** 1994. Effect of halothane on BK channels in cerebrovascular smooth muscle cells of adult rats. Anesthesiology **81:**649–656.
19. **Jarvis, K. G., J. A. Giron, A. E. Jerse, T. K. McDaniel, M. S. Donnenberg, and J. B. Kaper.** 1995. Enteropathogenic *Escherichia coli* contains a specialized secretion system necessary for the export of proteins involved in attaching and effacing lesion formation. Proc. Natl. Acad. Sci. USA **95:**7996–8000.
20. **Jerse, A. E., and J. B. Kaper.** 1991. The *eae* gene of enteropathogenic *Escherichia coli* encodes a 94-kilodalton membrane protein, the expression of which is influenced by the EAF plasmid. Infect. Immun. **59:**4302–4309.
21. **Kaper, J. B.** 1994. Molecular pathogenesis of enteropathogenic *Escherichia coli*, p. 173–195. *In* V. L. Miller, J. B. Kaper, D. A. Portnoy, and R. R. Istery (ed.), Molecular genetics of bacterial pathogenesis. American Society for Microbiology, Washington, D.C.
22. **Kenny, B., and B. B. Finlay.** 1995. Protein secretion by enteropathogenic *Escherichia coli* is essential for transducing signals to epithelial cells. Proc. Natl. Acad. Sci. USA **95:**7991–7995.
23. **Khoshoo, V., M. K. Bhan, M. Mathur, and P. Raj.** 1988. A fatal severe enteropathy associated with enteropathogenic *E. coli*. Indian Pediatr. **25:** 308–309.
24. **Knutton, S., T. Baldwin, P. H. Williams, and A. S. McNeish.** 1989. Actin accumulation at sites of bacterial adhesion to tissue culture cells: basis of a new diagnostic test for enteropathogenic and enterohemorrhagic *Escherichia coli*. Infect. Immun. **57:**1290–1298.
25. **Levine, M. M., E. J. Bergquist, D. R. Nalin, D. H. Waterman, R. B. Hornick, C. R. Young, S. Sotman, B. Rowe.** 1978. *Escherichia coli* strains that cause diarrhoea but do not produce heat-labile or heat-stable enterotoxins and are non-invasive. Lancet **i:**1119–1122.
26. **Levine, M. M., and R. Edelman.** 1984. Enteropathogenic *Escherichia coli* of classic serotypes associated with infant diarrhea: epidemiology and pathogenesis. Epidemiol. Rev. **6:**31–51.
27. **Missiaen, L., H. De Snedt, J. B. Pary, M. Oike, and R. Casteels.** 1994. Kinetics of empty store-activated Ca^{2+} influx in HeLa cells. J. Biol. Chem. **269:**5817–5823.
28. **Nicotera, P., and S. Orrenius.** 1992. Ca^{2+} and cell death. Ann. N. Y. Acad. Sci. **618:**17–27.
29. **Rosenshine, I., M. S. Donnenberg, J. B. Kaper, and B. B. Finlay.** 1992. Signal transduction between enteropathogenic *Escherichia coli* (EPEC) and epithe-

lial cells: EPEC induces tyrosine phosphorylation of host cell proteins to initiate cytoskeletal rearrangement and bacterial uptake. EMBO J. **11:**3551–3560.
30. **Rosenshine, I., and B. B. Finlay.** 1993. Exploitation of host signal transduction pathways and cytoskeletal functions by invasive bacteria. Bioessays **15:** 17–24.
31. **Rosenshine, I., S. Ruschkowski, and B. B. Finlay.** 1996. Expression of attaching/effacing activity by enteropathogenic *Escherichia coli* depends on growth phase, temperature, and protein synthesis upon contact with epithelial cells. Infect. Immun. **64:**966–973.
32. **Rosenshine, I., S. Ruschkowski, M. Stein, D. J. Reinscheid, S. D. Mills, and B. B. Finlay.** 1996. A pathogenic bacterium triggers epithelial signals to form a functional bacterial receptor that mediates actin pseudopod formation. EMBO J. **15:**2613–2624.
33. **Sakmann, B., and E. Neher.** 1983. Single-channel recording. Plenum Press, New York.
34. **Sauve, R., G. Roy, and D. Payet.** 1983. Single channel K^+ currents from HeLa cells. J. Membr. Biol. **74:**41–49.
35. **Sauve, R., C. Simmoneau, R. Monette, and G. Roy.** 1986. Single-channel analysis of the potassium permeability in HeLa cancer cells: evidence for a calcium-activated potassium channel of small unitary conductance. J. Membr. Biol. **92:**269–282.
36. **Sauve, R., C. Simmoneau, L. Parent, R. Monette, and G. Roy.** 1987. Oscillatory activation of calcium-dependent potassium channels in HeLa cells induced by histamine H_1 receptor stimulation: a single channel study. J. Membr. Biol. **96:**199–208.
37. **Spitz, J., R. Yuhan, A. Koutsouris, C. Blatt, J. Alverdy, and G. Hecht.** 1995. Enteropathogenic *Escherichia coli* adherence to intestinal epithelial monolayers diminishes barrier function. Am. J. Physiol. **268:**G374–G379.
38. **Takeyasu, K., M. M. Tamkun, K. J. Renaud, and D. M. Fambrough.** 1988. Ouabain-sensitive ($Na^+ + K^+$)-ATPase activity expressed in mouse L cells by transfection with DNA encoding the α-subunit of an avian sodium pump. J. Biol. Chem. **263:**4347–4354.
39. **Taylor, C. J., A. Hart, R. M. Batt, C. McDougall, and L. McLean.** 1986. Ultrastructural and biochemical changes in human jejunal mucosa associated with enteropathogenic *Escherichia coli* (O111) infection. J. Pediatr. Gastroenterol. Nutr. **5:**70–73.

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