Adrenergic modulation of *Escherichia coli* O157:H7 adherence to the colonic mucosa

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The human intestinal tract encompasses an extensive surface area ~300–400 m², which constitutes the largest interface between the host and microorganisms. It is colonized by at least 400 species of commensal bacteria at densities reaching 10¹¹ organisms/ml of luminal fluid (29). The intestine has developed a diverse array of innate protective mechanisms that allow it to coexist benignly with resident flora, yet effectively remove pathogenic microorganisms (12). Enterohemorrhagic *Escherichia coli* O157:H7 (EHEC) is a highly virulent enteric pathogen that is acquired by ingestion of contaminated food or water (2). The microorganism can produce acute gastroenteritis and severe hemorrhagic colitis, and its expression of bacteriophage-associated Shiga toxins is associated with the hemolytic uremic syndrome, which can lead to acute renal failure (35). One target for EHEC infection is the colonic mucosa. After its initial “loose” adherence to colonic surface cells, EHEC alters the epithelial actin cytoskeleton by delivering virulence proteins into host cells through a type III secretion system and produces characteristic attaching and effacing lesions (16). EHEC also targets the follicle-associated epithelium of Peyer’s patches in the small intestine (11, 27).

Neurons containing norepinephrine (NE) are located in prevertebral sympathetic ganglia located in the lumbar portion of the spinal cord. They send fiber projections to intramural neurons and nonneuronal target cells within the wall of the distal colon (14). Through interactions with α- and β-adrenergic receptors, NE modulates intestinal smooth muscle contractility, submucosal blood flow, and active transepithelial ion transport (24). Extrinsic sympathetic input to the gut mucosa may function to regulate adaptive immune responses to luminal antigens as well (10). NE concentrations in the intestinal lumen increase in response to surgery and other stressors and play an important role in the early stages of sepsis (1, 18). At high concentrations, NE has been shown to directly enhance the growth rate of commensal *E. coli* as well as the growth and virulence characteristics of EHEC (20–22, 30). Although enteroendocrine cells and hormones can affect active ion transport and other putative biodefensive functions of the intestinal mucosa, their role in modulating mucosal interactions with enteropathogens has not been well-defined (31).

We tested the hypothesis that NE plays a role in modulating interactions between EHEC and the colonic epithelium. The porcine distal colon was chosen as a biomedical model because it is functionally homologous to the human colon (33) and is susceptible to infection by a number of human enteropathogens, including EHEC (26). Moreover, NE transiently increases active chloride secretion through interactions with colonocyte α₁-adrenergic receptors in this tissue (37). In the present study, we examined the ability of NE to alter EHEC adherence to the colonic epithelium through a mechanism of action independent of mucosal ion transport.
Bacterial strains and growth conditions. Bacteria were stored in 20% (vol/vol) glycerol/PBS until time of culture. An inoculum of Escherichia coli O157:H7 [EHEC, Shiga toxin-negative strain 700728 American Type Culture Collection (Manassas, VA) or the Shiga toxin-producing strain EDL933] or a nonpathogenic, rodent-adapted E. coli (streptomycin-resistant strain M-21; Ref. 40) were grown overnight in Luria-Bertani (LB) medium at 37°C in a humidified 5% CO2 atmosphere. Shiga toxins produced by EHEC have been reported to induce apoptosis in epithelial cells; therefore, most experiments were carried out by using a Shiga toxin-negative strain of E. coli O157:H7 (strain 700728) to eliminate this potential variable (15). Inocula were diluted 1:9 in PBS and added to the luminal bathing medium. Strain 700728 EHEC and E. coli M-21 were used at luminal inocula of 5.96 × 105 ± 2.81 × 105 colony forming units (CFU/ml) and 1.52 × 106 ± 1.23 × 106 CFU/ml, respectively, as determined by spread plating.

Commensal porcine non-O157 E. coli was obtained by plating homogenized colonic mucosa from normal pigs onto Fluorocult agar supplemented with 100 μg/ml streptomycin sulfate. The selective isolation and differentiation capabilities of Fluorocult medium for Enterobacteriaceae, especially E. coli O157:H7, which are achieved by a combination of fluorescent and chromogenic substrates, have been well described to identify relevant bacteria from a variety of sources (13, 23). Presumptive colonies of E. coli that did not have the appearance of E. coli O157:H7 were randomly chosen from Fluorocult plates after overnight incubation and were streaked onto LB agar plates supplemented with 100 μg/ml streptomycin to isolate E. coli strains resistant to this antibiotic drug. After 24-h incubation at 37°C, individual colonies were picked from streptomycin sulfate-supplemented LB plates, and their identities were confirmed as E. coli using the API-20E Enteric Identification System (BioMerieux, Hazelwood, MO). Colonies were further determined to represent non-O157 E. coli with the use of an E. coli O157 latex agglutination-based diagnostic test kit (Oxoid, Ogdenburg, NY).

Drugs. Rp-8-bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp-8-Br-cAMPs) and Sp-8-bromoadenosine-3′,5′-cyclic monophosphorothioate (Sp-8-Br-cAMPS) were obtained from Alexis Biochemicals (San Diego, CA). All other drugs were obtained from Sigma (St. Louis, MO). Yohimbine and prazosin were dissolved in methanol. Phosphorylated cyclic nucleotides (Sp-8-Br-cAMPS) and Sp-8-bromoadenosine-3′,5′-cyclic monophosphorothioate (Rp-8-Br-cAMPS) were obtained from Alexis Biochemicals (San Diego, CA). All other drugs were obtained from Sigma (St. Louis, MO). Yohimbine and prazosin were dissolved in methanol. The other drugs were solubilized in physiological saline solution (composition indicated in Tissue preparation and measurement of transepithelial ion transport) as concentrated stock solutions and stored at −80°C; NE and propranolol solutions were prepared immediately before use.

Agonists were added to the luminal or contraluminal media bathing colonic mucosal sheets 5 min before bacterial exposure; adrenergic receptor antagonists were added to the contraluminal bathing medium 10 min before agonist addition. When given alone, Rp-8-Br-cAMPS and Sp-8-Br-cAMPS were added to the contraluminal bathing medium 5 min before EHEC exposure; in NE experiments, Sp-8-Br-cAMPS was added 10 min before addition of NE.

Animals. Tissues were obtained from outbred Yorkshire/Landrace-crossed pigs of each sex that were 5–9 wk old and weighed between 10 and 18 kg. Pigs had continuous access to water and nonmedicated feed and were not fasted before death. They were sedated with an intramuscular injection of tiletamine hydrochloride-zolazepam (Tela- zol; 8 mg/kg, Fort Dodge Laboratories, Fort Dodge, IA), in combination with xylazine (3 mg/kg). They were subsequently euthanized by barbiturate overdose in accordance with approved University of Minnesota Institutional Animal Care and Use Committee protocols. A midline laparotomy was performed to expose the intestine, and a 7-cm segment of the distal colon extending orad from the internal anal sphincter was isolated.

Tissue preparation and measurement of transepithelial ion transport. The colonic epithelium was stripped of underlying smooth muscle coats, and the mucosa with attached submucosa was mounted in Ussing chambers (1- or 2-cm2 flux area). The serosa and smooth muscle layers of an excised colonic segment were removed by blunt dissection, and the remaining submucosa-mucosa was mounted between two Lucite Ussing-type half chambers. Mucosal sheets were bathed on both luminal and contraluminal aspects in 10 ml of a buffered, physiological saline solution (composition in mM: 130 NaCl, 6 KCl, 3 CaCl2, 0.7 MgCl2, 20 NaHCO3, 0.29 NaH2PO4, and 1.3 NaHPO4) that was continuously oxygenated with 95% O2-5% CO2 delivered by gas lift and maintained at pH 7.4 and 39°C (porcine core temperature). D-Glucose and mannitol (10 mM) were added to the contraluminal and luminal bathing media, respectively.

Short-circuit current (Isc, in μA/cm2) was monitored continuously across each mucosa-submucosal sheet with an automatic voltage clamp apparatus (model TR100; JWT Engineering, Overland Park, KS or model EVC-4000; World Precision Instruments, Sarasota, FL). Experiments were initiated after the basal Isc had stabilized (25–35 min). Throughout each experiment, the transepithelial voltage was periodically adjusted to 5 mV, and the resulting current change was used to calculate the tissue conductance (Gt) by Ohm’s law. Isc was measured immediately before drug administration and at the peak of drug action.

Bacterial adherence and gentamicin resistance assays. Bacterial adherence to the colonic mucosa was determined by the method of Knutton et al. (17). Mucosal sheets were removed from Ussing chambers after 90 min of luminal exposure to bacteria. Each tissue was weighed and washed three times in PBS (pH 7.4). In some cases, tissues were divided in half, weighed, and assayed for both bacterial adherence and bacterial internalization. All tissues were subsequently homogenized by using a Brinkmann Polytron and spread-plated on differential and selective media for O157:H7 (Fluorocult agar, EM Science, Gibbstown, NJ) and M-21 (MacConkey agar; Difco, Detroit, MI, containing 1 mg/ml streptomycin sulfate). For experiments that examined the ability of commensal non-O157 E. coli obtained from pigs as described above in Bacterial strains and growth conditions to adhere to the colonic mucosa, homogenates were plated on Fluorocult agar supplemented with 100 μg/ml streptomycin sulfate.

To assess the intracellular invasion of EHEC, colonic sheets removed from Ussing chambers after 90 min of EHEC exposure were incubated at 37°C in a humidified 5% CO2 atmosphere in a gentamicin solution (100 μg/ml in PBS) for 80 min to eliminate extracellular bacteria after the method of Elingshorst (6). The tissues were subsequently homogenized and spread-plated in a manner identical to that performed in bacterial adherence assays.

Visualization and quantitation of bacterial adherence. Bacterial aggregates immunoreactive for the O-antigen of E. coli O157:H7 that adhered to whole mounts of the colonic mucosa obtained from seven pigs were examined and quantified by confocal laser-scanning microscopy after luminal exposure to E. coli O157:H7 in Ussing chambers. Some tissues were unexposed to bacteria or pretreated contraluminal media with 10 μM NE in the absence or presence of 1 μM phentolamine. Mucosal sheets were removed from the chambers after 90 min of EHEC exposure and gently washed three times in PBS (pH 7.4). EHEC on the colonic mucosa was visualized and quantified by immunofluorescence. Briefly, whole mounts of colonic sheets were incubated in PBS containing 2.5% bovine serum albumin (Sigma) for 30 min at room temperature on an orbit shaker. Whole mounts were then incubated for 2 h at room temperature with a FITC-conjugated goat anti-E. coli O157:H7 affinity-purified antibody directed toward the O-antigen of E. coli O157:H7 (1:20 dilution; BacTrace antibody; Kirkegaard and Perry Laboratories, Gaithersburg, MD). After brief washes in PBS, whole mounts were incubated for 30 min at room temperature with Texas Red-conjugated antibody to DNAse (Molecular Probes, Eugene, OR) to visualize the colonic surface. After
additional washes in PBS, whole mounts were fixed in 2% paraformaldehyde (Sigma) for 1 h and refrigerated in PBS until visualized. Images from five randomly selected, nonoverlapping fields, each representing a total visual field of 1.5 mm², were acquired by using Comos software (version 6.05.8; Comos Bio-Rad, Hercules, CA) and further processed employing NIH Image software (version 1.6.0) and Adobe Photoshop (version 6.0.1, Adobe Systems, San Jose, CA). An

Table 1. Time-related changes in the electrical properties of colonic tissues exposed to luminal EHEC in the absence and presence of 10 μM norepinephrine in contraluminal bathing medium

<table>
<thead>
<tr>
<th>Condition</th>
<th>n/N</th>
<th>I_sc (μA/cm²)</th>
<th>G_t (mS/cm²)</th>
<th>I_sc (μA/cm²)</th>
<th>G_t (mS/cm²)</th>
<th>I_sc (μA/cm²)</th>
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<th>I_sc (μA/cm²)</th>
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<td>12±1</td>
<td>-5±1</td>
<td>13±1</td>
<td>1±5</td>
<td>14±1</td>
<td>1±4</td>
<td>24±2*</td>
</tr>
<tr>
<td>NE‡</td>
<td>50/38</td>
<td>12±5</td>
<td>14±1</td>
<td>2±11</td>
<td>16±1</td>
<td>12±7</td>
<td>15±1</td>
<td>10±5</td>
<td>25±2*</td>
</tr>
</tbody>
</table>

Mean short-circuit current (I_sc) and tissue electrical conductance (G_t) values are expressed as μA/cm² and mS/cm², respectively. n/N, number of tissues from number (N) of pigs; t, time. †Time elapsed after luminal addition of Escherichia coli O157:H7, strain 700728; ‡added to the contraluminal bathing medium at t = -5 min. *P < 0.05 vs. t = 0 min, Dunnett’s test. EHEC, enterohemorrhagic Escherichia coli.

Fig. 1. Concentration-effect relationship for the actions of norepinephrine (NE) in the porcine colonic mucosa. A: effects of NE on enterohemorrhagic Escherichia coli O157:H7 (EHEC) adherence. B: effects of NE on short-circuit current (I_sc). Data represent means ± SE of counts of adherent EHEC [in colony forming units (CFU)/g; 6–19 tissues from 6–18 pigs] or peak changes in I_sc (in μA/cm²; 6–42 tissues from 6–39 pigs) measured after the contraluminal addition of NE at the log₁₀ molar concentrations indicated.

Fig. 2. Effects of phentolamine (PTL) or saxitoxin (STX) on NE-induced EHEC adherence. A: effects of NE on the adherence of E. coli O157 (solid bars) or E. coli M-21 (patterned bars) to porcine colonic mucosa. Tissues were untreated (control), treated with 10 μM NE, or treated with 1 μM PTL (PTL/NE) before administration of NE. Bars represent the means ± SE counts of adherent EHEC (in CFU/g) in 14 control tissues (from 8 pigs), 12 tissues (from 8 pigs) treated with NE, and 16 tissues (from 8 pigs) treated with PTL and NE. In 1 animal, a tissue serving as a control had 259,176 EHEC CFU/g compared with a mean of 10,874 ± 3,037 CFU/g for 14 other tissues serving as controls. Data obtained in all tissues from this animal were excluded from further analysis. The mean counts of adherent M-21 (in CFU/g) were obtained in 6 control tissues (from 3 pigs) and 6 tissues (from 3 pigs) treated with NE. B: effects of STX on the action of NE. Tissues were untreated (control), treated with 0.1 μM STX before administration of 10 μM NE, or treated with 0.1 μM STX alone. Bars represent the means ± SE counts of adherent EHEC (in CFU/g) in 14 control tissues (from 9 pigs), 7 tissues (from 7 pigs) treated with both STX and NE, and 6 tissues (from 4 pigs) treated with STX alone. *P < 0.05 vs. EHEC adherence in control tissues, #P < 0.05 vs. control tissues exposed to EHEC, as determined by unpaired t-tests with Welch’s correction.

Mean short-circuit current (I_sc) and tissue electrical conductance (G_t) values are expressed as μA/cm² and mS/cm², respectively. n/N, number of tissues from number (N) of pigs; t, time. †Time elapsed after luminal addition of Escherichia coli O157:H7, strain 700728; ‡added to the contraluminal bathing medium at t = -5 min. *P < 0.05 vs. t = 0 min, Dunnett’s test. EHEC, enterohemorrhagic Escherichia coli.
Bio-Rad) attached to a Nikon microscope. The different fluorophores were imaged sequentially. Images were acquired and processed as described above.

Data analysis. Data are expressed as means ± SE of colony forming units per gram of tissue, \( I_{sc} \) (in \( \mu A/cm^2 \)), \( G_i \) (in millisiemens/cm²) or area encompassed by adherent immunoreactive EHEC per tissue in square pixels. Statistical analyses of data were performed by using the PRISM computer software program (Version 3.0; GraphPad Software, San Diego, CA). Comparisons between a control mean and a single treatment mean were made by unpaired \( t \)-tests with Welch’s correction used for unequal variances. Comparisons of a control mean with multiple treatment means were made by one-way or two-way ANOVA followed by Dunnett’s test where appropriate. In all cases, the limit for statistical significance was set at \( P < 0.05 \).

**RESULTS**

**Baseline electrical parameters.** Under baseline conditions, \( I_{sc} \) and \( G_i \) in uninfected, isolated sheets of colonic mucosa-submucosa averaged 2 ± 2 \( \mu A/cm^2 \) and 15 ± 1 mS/cm², respectively (\( n = 405 \) tissues from 37 pigs). At 90 min, \( I_{sc} \) remained unchanged in EHEC-infected tissues, but \( G_i \) increased relative to earlier time points. However, this increase in \( G_i \) was similar in untreated tissues serving as controls and tissues treated with NE (Table 1). The contraluminal addition of NE at 10 \( \mu M \) produced a rapid elevation in \( I_{sc} \) with a mean duration of 46 ± 8 min before return to baseline values (\( n = 8 \) tissues from 4 pigs); at 90 min, all NE-treated tissues had returned to baseline \( I_{sc} \) (Table 1). The luminal addition of *E. coli* O157:H7 strain 700728 did not produce a significant, acute change in either \( I_{sc} \) or \( G_i \).

![Figure 3](http://ajpgi.physiology.org/)

**Fig. 3.** Effect of selective \( \alpha \)-adrenergic antagonists on NE-induced EHEC adherence. Tissues were either untreated (control), treated with 10 \( \mu M \) NE, or treated with 0.3 \( \mu M \) prazosin (PRZ/NE) or yohimbine (YHB/NE) before NE administration. The antagonists and NE were added to the contraluminal bathing medium 15 and 5 min, respectively, before luminal exposure to EHEC in the stationary growth phase. Bars represent the means ± SE counts of adherent EHEC (in CFU/g) in 12 control tissues (from 7 pigs), 8 tissues (from 8 pigs) treated with NE, 8 tissues (from 8 pigs) treated with PRZ and NE, and 8 tissues (from 8 pigs) pretreated with YHB/NE. *P < 0.05 vs. control mean, Dunnett’s test.

![Figure 4](http://ajpgi.physiology.org/)

**Fig. 4.** The effect of PKA modulators on EHEC adherence to the porcine colonic mucosa. Tissues were untreated (control) or pretreated contraluminally with either the PKA inhibitor Rp-8-bromoadenosine-3’,5’-cyclic monophosphorothioate (Rp-8-Br-cAMPS; 3 \( \mu M \)) or the PKA activator Sp-8-Br-cAMPS alone, 10 tissues (from 8 pigs) treated with Sp-8-Br-cAMPS alone, and 6 tissues (from 3 pigs) treated with Sp-8-Br-cAMPS and NE. *P < 0.05 vs. control mean, Dunnett’s test.
Concentration-dependent actions of NE on interactions of the colonic mucosa with EHEC. EHEC strain 700728 adhered to, but did not substantially invade the colonic mucosa. After a 90-min period of EHEC exposure, the number of bacteria internalized in and adhering to the mucosa under control conditions was $45 \pm 11$ and $5,779 \pm 1,369$ CFU/g tissue, respectively ($n = 11$ tissues from 7 pigs). The contraluminal addition of NE over a concentration range of $0.1–10 \mu M$ increased luminal adherence of the toxin-negative EHEC strain to sheets of colonic mucosa in a concentration-dependent fashion (Fig. 1). The contraluminal concentrations of NE producing EC$_{50}$ on EHEC adherence and $I_{sc}$ were significantly different (EC$_{50}$ for increasing adherence and $I_{sc}$ were 1.32 and 0.82 $\mu M$ with 95% confidence limits of 1.27–1.37 and 0.74–0.91 $\mu M$, respectively). However, NE at a contraluminal concentration of $10 \mu M$ had no effect on EHEC internalization ($31 \pm 21$ CFU/g recovered from 5 gentamicin-treated tissues from 3 pigs). When added to the luminal bathing medium, $10 \mu M$ NE had no significant effect on EHEC adherence ($8,678 \pm 1,875$ and $11,541 \pm 1,791$ CFU/g, respectively, in 15 control and 14 NE-treated tissues from 9 pigs; $P > 0.05$, t-test).

NE also significantly increased mucosal adherence of Shiga toxin-positive E. coli O157:H7 strain EDL933 added in an inoculum of $4,820 \pm 3,191$ CFU/ml of luminal bathing medium. Bacterial adherence was $10,973 \pm 2,737$ and $89,034 \pm 10,113$ CFU/g in tissues untreated or contraluminally pre-treated with $10 \mu M$ NE, respectively ($P < 0.05$, t-test, $n = 3$ and 4 tissues respectively, from 3 pigs). Two streptomycin-resistant strains of non-0157:H7 E. coli were isolated from porcine colon and grown in a manner similar to E. coli O157:H7 also adhered to the colonic mucosa, but NE in the contraluminal bathing medium did not significantly change mucosal adherence of these organisms (number of colonic non-EHEC E. coli strain no. 1 in the absence and presence of $10 \mu M$ NE = $2,333 \pm 928$ and $5,464 \pm 1,099$ CFU/g tissue, respectively; $P > 0.05$, unpaired t-test, 4 control and NE-treated tissues from 4 pigs; number of colonic non-EHEC E. coli strain no. 2 in the absence and presence of $10 \mu M$ NE =

Fig. 5. Visualization of EHEC-like immunoreactivity on the luminal surface of porcine colonic mucosa. A: whole mount of colonic mucosal surface that was not exposed to EHEC. B: whole mount of mucosal surface exposed to EHEC for 90 min but untreated with drugs. Immunoreactive microcolonies (green) can be seen. C: mucosal surface of a tissue pretreated contraluminally with $10 \mu M$ NE 5 min before luminal EHEC exposure for 90 min. A relative increase in the number of adherent microcolonies compared with B can be observed. D: mucosal surface of a tissue pretreated contraluminally with 1 $\mu M$ PTL and $10 \mu M$ NE and subsequently exposed to EHEC for 90 min. Note a similar number of microcolonies as seen in B and a lesser number than that seen in C. Bar = 50 $\mu m$. E: quantitation of EHEC immunoreactivity adhering to the mucosal surfaces of colonic sheets from 6 pigs. Values represent the mean area occupied by adherent, immunoreactive EHEC per tissue in square pixels as determined in each of 5 nonoverlapping visual fields of 1.5 mm$^2$ area per treatment condition in tissues from 6 pigs (*$P < 0.05$ vs. control mean, Dunnett’s test).
ergic antagonist prazosin (Fig. 3). However, yohimbine did not alter the ability of NE to increase $I_{sc}$ ($\Delta I_{sc} = 82 \pm 10 \mu A/cm^2$ in 46 NE-treated tissues from 38 pigs and 104 $\pm 27 \mu A/cm^2$ in 8 tissues treated both with yohimbine and NE from 8 pigs).

Yohimbine-sensitive $\alpha_2$-adrenergic receptors are coupled through the GTP-binding protein $G_{\alpha_i}$ to decreases in intracellular cAMP production and secondary decreases in protein kinase A (PKA) activity. To test the hypothesis that direct inhibition of PKA activity would mimic the action of NE on EHEC adherence, mucosal sheets were treated contraluminally with either $3 \mu M$ Rp-8-Br-cAMPS or Sp-8-Br-cAMPS before luminal EHEC exposure. These membrane-permeant cAMP analogs inhibit and stimulate PKA activity, respectively (9). As shown in Fig. 4, pretreatment with Rp-8-Br-cAMPS, but not Sp-8-Br-cAMPS increased EHEC adherence. However, Sp-8-Br-cAMPS pretreatment appeared to prevent the action of NE on bacterial adherence (Fig. 4). Neither PKA modulator had significant effects on baseline $I_{sc}$ or $G_i$ (data not shown).

Moreover, the mucosal $I_{sc}$ response to NE in tissues pretreated with Sp-8-Br-cAMPS was not significantly different from tissues treated with $10 \mu M$ NE alone ($\Delta I_{sc} = 82 \pm 10 \mu A/cm^2$ in 46 NE-treated tissues from 38 pigs, and $66 \pm 25 \mu A/cm^2$ in 6 Sp-8-Br-cAMPS- and NE-treated tissues from 3 pigs, $P > 0.05$, t-test).

Visualization of adherent EHEC in NE-pretreated and untreated colonic sheets. Whole mounts of mucosal sheets serving as controls that were not exposed to EHEC did not exhibit...
immunoreactivity toward the O-antigen of *E. coli* O157:H7 (Fig. 5A). In contrast, tissues exposed to luminal EHEC displayed numerous immunoreactive aggregates that appear to represent *E. coli* O157:H7 microcolonies (Fig. 5, B–D). Sheets of colonic mucosa treated contraluminally with 10 μM NE before luminal EHEC exposure manifested a greater number of immunoreactive aggregates (Fig. 5, C and E) than those either not treated with NE or pretreated with 1 μM phenotolamine before NE administration (Fig. 5, B, D, and E).

**Immunohistochemical localization of noradrenergic nerve fibers in porcine colonic mucosa and modulation of EHEC adherence by endogenous NE.** Nerve fibers immunoreactive for the NE-synthesizing enzymes TH and DBH were observed in the submucosal plexuses and mucosa of the porcine distal colon (Fig. 6A) and throughout the myenteric plexus and circular muscle layer (data not shown). There was frequent colocalization of TH- and DBH-immunoreactivities in colonic nerve fibers (Figs. 6, B–D). Fine, varicose nerve fibers exhibiting both TH and DBH immunoreactivities were observed throughout the colonic villi. They were often seen terminating near colonic epithelial cells (Fig. 6A).

To test the hypothesis that endogenous NE is capable of increasing the epithelial adherence of EHEC, tissues were pretreated with the NE reuptake blocker desipramine and the monoamine oxidase inhibitor pargyline. These drugs in combination mimicked the effect of NE on EHEC adherence (Fig. 7). The contraluminal addition of the NE-releasing agent tyramine did not further increase EHEC adherence (Fig. 7). The effect of these drugs on EHEC adherence was abolished in tissues pretreated with phenotolamine (Fig. 7). The three sympathomimetic agents in combination did not produce significant changes in $I_{sc}$ or $G_{t}$ (data not shown).

**DISCUSSION**

Pigs have been used previously as an animal model for studies of *E. coli* O157:H7 infections (4, 26, 38). The ability of enteric neurotransmitters, such as NE, to modulate mucosal interactions with EHEC and other pathogens has not been examined previously in the pig. The results presented demonstrate that the catecholamine NE significantly increased EHEC adherence to muscle-stripped sheets of porcine distal colonic mucosa. NE has previously been shown to transiently increase $I_{sc}$ in this tissue. This effect has been attributed to an increase in net chloride secretion and appears to be mediated by prazosin-sensitive, $\alpha_1$-adrenergic receptors (37). In the present investigation, NE increased both $I_{sc}$ and the adherence of Shiga toxin-positive and –negative strains of *E. coli* O157:H7 to the colonic mucosa. The potency of NE in elevating $I_{sc}$ was 1.6-fold greater than its potency in promoting the adherence of EHEC, and both effects were resistant to the axonal conduction blocker saxitoxin, indicating that they may result from direct interactions of NE with colonocytes rather than through indirect actions through enteric neurons. The $\alpha_2$-adrenergic antagonist yohimbine inhibited the effects of NE on EHEC adherence. Moreover, by acting downstream of $\alpha_2$-adrenergic receptors, the PKA activator Sp-8-Br-cAMPS appeared to antagonize the action of NE on bacterial adherence. In contrast, mucosal $I_{sc}$ responses to NE were resistant to yohimbine, and PKA modulators had no effect on colonic ion transport. Therefore, it appears that the effect of NE on EHEC adherence is mediated by $\alpha_2$-adrenergic receptors that are negatively coupled to PKA activity in colonocytes. On the basis of these results, we hypothesize that NE increases active ion transport and bacterial adherence in colonic epithelial cells through different mechanisms and cellular sites of action.

NE increased EHEC adherence after its addition to the contraluminal, but not luminal bathing medium. This finding is consistent with a site of action for the catecholamine at the basolateral aspect of the colonic epithelium and indicates that NE does not interact directly with EHEC during the relatively short 90-min duration of the assay. Indeed, a previous report (21) indicated that a minimum time period of 4 to 6 h is required for NE to directly influence EHEC growth and virulence. The relatively short time course of NE action suggests that the catecholamine may be promoting the initial loose adherence of EHEC to the mucosa, rather than EHEC attachment and effacement. Much less is known about the process of initial EHEC adherence compared with attachment and effacement. Recently, a fimbral operon of *E. coli* O157:H7 has been discovered, which appears to play a role in microcolony formation and bacterial interactions with epithelial cells (36). Moreover, flagella of the closely related enteropathogenic *E. coli* appear to mediate adherence of this organism to epithelial cells and flagellum production may be induced by host signals (8).

We hypothesize that NE-mediated decreases in PKA activity may modulate the expression of host protein(s) mediating the process of initial EHEC adherence to the mucosa. Although a phenomenon such as this has not hitherto been reported, NE has been shown to modulate the expression of...
intercellular adhesion molecule-1 in leukocytes through a β2-adrenergic receptor-mediated mechanism (34).

The ability of NE to promote the adherence of both Shiga toxin-positive and -negative strains of E. coli O157:H7 indicates that the expression of these exotoxins is not an important factor in EHEC adherence. Moreover, NE appeared to selectively modulate the adherence of pathogenic E. coli, because it had no effect on either the adherence of nonpathogenic rodent- or porcine-adapted non-0157/ strains of E. coli. The rodent-adapted commensal E. coli M-21 has been shown previously to be internalized by Caco-2 cells at counts similar to those determined by gentamicin resistance assay in the present study

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vate the colonic mucosa. These fibers probablyemanate from neurons in the caudal mesenteric ganglion as almost 85% of TH-immunoreactive cells in this ganglion project to the porcine colon and rectum (28). A combination of the indirectly acting sympathomimetic agents desipramine, pargyline, and tyramine mimicked the action of NE on EHEC adherence, but did not affect $I_{sc}$. Moreover, the α-adrenergic antagonist phentolamine prevented the effects of exogenous NE and the sympathomimetic agents. These data suggest that NE present in adrenergic nerve fibers innervating the colonic mucosa may be released in quantities sufficient to increase EHEC adherence through an action on colonocyte α-adrenergic receptors, particularly in tissues treated with NE reuptake and degradation inhibitors. The inability of the indirectly acting sympathomimetic drugs to alter $I_{sc}$ might be attributed to the predominant localization of TH/DBH-immunoreactive nerve fibers near colonic surface cells rather than in the colonic crypts, a site for active chloride secretion.

The enteric nervous system appears to modulate epithelial defense and repair processes associated with intestinal infec-

tion (31). However, little is known concerning the effects of enteric neurotransmitters and hormones on the interactions between microbes and the intestinal mucosa. Because NE also increases adherence of EHEC to the cecal mucosa of mice (3), the phenomenon investigated here appears not to be restricted to the colon or to a particular animal species. We hypothesize that changes in sympathetic neural outflow to the colon may increase susceptibility of the host to infection by enteropathogens such as EHEC.

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