Modulation of Pathogenicity with Norepinephrine Related to the Type III Secretion System of *Vibrio parahaemolyticus*

Masayuki Nakano, Akira Takahashi, Yuko Sakai, and Yutaka Nakaya

Department of Nutrition and Metabolism, Institute of Health Biosciences, University of Tokushima, Tokushima, Japan

(See the editorial commentary by Waldor and Sperandio, on pages 1248–9.)

**Background.** Norepinephrine (NE) controls the functions of the gastrointestinal tract, but its role in the pathogenicity of enteropathogens is not clear. We examined the effect of NE on the pathogenicity of *Vibrio parahaemolyticus* with regard to its type III secretion systems (TTSSs).

**Methods.** To evaluate the effect of NE on pathogenicity of *V. parahaemolyticus*, we examined the cytotoxic activity to Caco-2 cells and enterotoxicity by use of the rat ileal loop model. It has been reported that TTSS1 causes cytotoxicity and that TTSS2 causes enterotoxicity in the animal ileal loop model.

**Results.** Our results showed that, although NE alone did not affect the viability of Caco-2 cells, NE stimulated the cytotoxic activity of *V. parahaemolyticus*. Furthermore, NE increased the transcription of the TTSS1-related genes vscQ and vscU. These results indicate that NE regulates *V. parahaemolyticus* cytotoxic activity. The enterotoxicity of *V. parahaemolyticus* was increased by NE through interaction with α1-adrenergic receptors. These results indicate that α1-adrenergic receptors on the intestinal epithelium appear to interact with *V. parahaemolyticus* enterotoxicity.

**Conclusions.** The present findings suggest that enteric NE may modulate *V. parahaemolyticus* pathogenicity.

Norepinephrine (NE) is not only a neurotransmitter in the central nervous system but is also known to modulate the functions of the gut, such as smooth muscle contractility, submucosal blood flow, and active transepithelial ion transport, through interactions with α- and β-adrenergic receptors [1]. Over one-half of all NE in the human body is produced from the epithelial cells in the gastrointestinal tract, and it seems likely that a large quantity of NE is present in the small intestine [2].

It has been reported that the influence of psychological and physical stress increases the symptoms of gastrointestinal diseases such as sepsis derived from the gut and that the release of NE from the intestinal epithelium is elevated in the intestinal lumen under stress exposure [3]. In particular, NE in the intestinal tract is considered to be the critical factor for early stage of sepsis [4, 5]. In addition, NE probably regulates adaptive immune responses to luminal antigens [6]. Recently, it has been demonstrated that the neuroendocrine hormone NE modulates the ability of enterohemorrhagic *Escherichia coli* (EHEC) to adhere to the colonic mucosa and stimulates its growth under in vitro and in vivo conditions [7–9]. Furthermore, NE stimulates the invasion of porcine jejunal explants by *Salmonella* and EHEC [10]. These results indicate that enteric NE may contribute to the pathogenicity of enteropathogens with clinical implications and that pathogenic gram-negative bacteria may have conserved strategies for sensing to host signal molecules.

*Vibrio parahaemolyticus* is a major causative agent of gastroenteritis, which is often associated with the consumption of raw or undercooked shellfish. This organism causes clinical manifestations such as gastroenter-
itis, wound infections, and septicemia [11–13]. Recently, the complete genome sequence of this organism revealed that the genes for the type III secretion systems (TTSSs) are present on the chromosome of *V. parahaemolyticus* [14]. TTSSs have been found in many types of pathogenic bacteria, including *Salmonella*, *Shigella*, *Yersinia*, and pathogenic *E. coli* [15]. It has been shown that TTSS contributes to bacterial pathogenicity via the injection of bacterial proteins directly into host target cells. A recent study has emphasized that TTSS1 is responsible for cytotoxic activity and that TTSS2 plays a role in enterotoxicity in rabbit ileal loop model [16]. Thus, TTSS1 and TTSS2 are considered to be important for the pathogenicity of *V. parahaemolyticus*. However, it is not clear how these virulence factors related to host molecules and clinical manifestations, such as NE effects on pathogenicity with regard to the TTSSs of *V. parahaemolyticus*.

In this article, we examine the ability of NE to alter the pathogenicity of *V. parahaemolyticus* and its relationship to TTSS1 and TTSS2 by measuring cytotoxic activity in a cultured cell line and the rat ileal loop model. We found that NE modulates both the cytotoxic activity and enteropathogenicity of *V. parahaemolyticus* via TTSS1 and TTSS2.

**MATERIALS AND METHODS**

**Bacterial culture conditions.** *V. parahaemolyticus* strain RIMD2210633 was used in this study [14]. The bacteria were routinely cultured in Luria-Bertani (LB) broth supplemented with 3% NaCl.

Quantitative real-time reverse-transcriptase polymerase chain reaction (RT-PCR) experiments with virulence-associated genes in the presence of cultured epithelial cells were performed as described elsewhere [17]. Each experiment was performed independently 5 times. The specificity of each reaction was monitored by the melting curve analysis.

Data collection was performed using Light Cycler software (version 3.5.3; Roche). Data were normalized to the levels of 16s rRNA and analyzed by use of the fit point method in accordance with the manufacturer’s instructions. Data analysis was performed as described elsewhere [17]. Each experiment was performed independently 5 times.

**Construction of deletion mutant strains.** The *vcrD1* and *vcrD2* deletion mutants, which had disrupted TTSS1 and TTSS2, respectively, were constructed as in previous reports [16, 18]. The target genes were disrupted by in-frame deletions via homologous recombination using a suicide vector.

**Cytotoxicity assay.** To examine *V. parahaemolyticus* cytotoxicity, the release of lactate dehydrogenase (LDH) was measured using a CytoTox96 nonradioactive cytotoxicity kit (Promega) in accordance with the manufacturer’s instructions [16, 18].

### Table 1. Primers for quantitative reverse-transcriptase polymerase chain reaction.

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer, 5’→3’</th>
<th>Temperature, °C</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>tdh</em></td>
<td>GTCCTGACTTTTGGACA ACATCTCACATGACTGTGA</td>
<td>60</td>
</tr>
<tr>
<td><em>rrs</em></td>
<td>CCTGCGAAGTGGACGGAATTCC GCCACCGTGAATACGTTCC</td>
<td>60</td>
</tr>
<tr>
<td><em>vopD</em></td>
<td>ACTGATGAGTACCTACGGCGA AGTGGTTTACAGCCGTATCG</td>
<td>60</td>
</tr>
<tr>
<td><em>vscQ</em></td>
<td>GGAAGTGGAAGTGCAAGCCGAA TGTGAGTTTGCAGAAGAGC</td>
<td>60</td>
</tr>
<tr>
<td><em>vscR</em></td>
<td>AGTGTGTTACAGCGCTATCG AGTGGTTTACAGCCGTATCG</td>
<td>64</td>
</tr>
<tr>
<td><em>vopA</em></td>
<td>CAAAAGGACGACGACTGC CAAGTAGAGTAGGAAGCCGA</td>
<td>64</td>
</tr>
<tr>
<td><em>vpa1321</em></td>
<td>ATGTAGTACGACGACTGC GGTGCTGGGCGATGTAGCA</td>
<td>64</td>
</tr>
<tr>
<td><em>vpa1327</em></td>
<td>ATGTGAGTTTGCAGAAGAGC AGTGGTTTACAGCCGTATCG</td>
<td>60</td>
</tr>
<tr>
<td><em>vopA</em></td>
<td>ATTGATAGCCTGGTGACTGC ATGTGAGTTTGCAGAAGAGC</td>
<td>60</td>
</tr>
</tbody>
</table>

1354  JID 2007:195 (1 May)  Nakano et al.
In brief, Caco-2 cells were washed with PBS and incubated further with fresh DMEM without phenol red (Sigma-Aldrich) before infection. At 8 h after infection, the supernatants were collected, and the release of LDH was quantified. The LDH release, which evaluates the percent cytotoxicity, was calculated with the following equation: (experimental release−spontaneous release)/(maximum release−spontaneous release)×100. The amounts of spontaneous and maximum release are the amount of LDH released from cytoplasm and released by total lysis of uninfected Caco-2 cells, respectively.

The bacterial cells were cultured in LB broth supplemented with 3% NaCl at 37°C for 15 h. After cultivation, bacterial cells were adjusted with PBS to OD_{600} = 1.0. The bacterial cells were resuspended in PBS at a 10^{-4}-fold dilution.

**Rat ileal loop assay.** The ileal loop model [19] was used to evaluate the effect of NE on intestinal fluid secretion after *V. parahaemolyticus* infection. The bacterial cells were cultured in LB medium supplemented with 3% NaCl at 37°C for 15 h with shaking. After cultivation, bacterial cells were harvested by centrifugation and resuspended in PBS (pH 7.4). The concentration was adjusted with PBS to OD_{600} = 1.0.

Albino Wister male rats (8 weeks old; 150–250 g) were fasted for 24 h but provided with water. Rats were anesthetized with sodium pentobarbital in accordance with the manufacturer’s instructions (Nembutal; Dainippon Sumitomo Pharma). Intestines were exposed by abdominal incision, and each loop was tied with thread. The ligated loops were ~5 cm in length and were separated from each other by 2-cm intervals. A bacterial suspension (0.5 mL; 2.31 ± 1.11 × 10^{8} cfu/mL) and agents were injected into each loop. The fluid content of each loop was measured 13 h after infection. The sample activities were measured and expressed as the ratio of the fluid content of the loop (in milliliters) to its length (in centimeters).

**Data analysis.** All data were expressed as mean ± SE values of the results for each reaction. Statistical significance was calculated by paired and unpaired t tests. In all cases, P < .01 was considered significant.

**RESULTS**

**Stimulation of the cytotoxic activity of *V. parahaemolyticus* by NE.** The cytotoxic activity of pathogenic bacteria is considered to be a good maker for their pathogenicity in humans [16]. To evaluate whether NE modulates the virulence of *V. parahaemolyticus*, we examined the effect of NE on cytotoxic activity toward Caco-2 cells. Before performing the infection assay, we examined the growth of *V. parahaemolyticus* strain RIMD2210633 in DMEM. The growth of *V. parahaemolyticus* in the presence of 50 μmol/L NE was almost identical to that in the absence of NE (generation time for 6–10 h after inoculation, 17.20 ± 1.23 min in the presence of NE and 17.43 ± 1.18 min in the absence of NE) (figure 1). This result indicates that NE does not affect the bacterial growth in DMEM.

The cytotoxic activity of *V. parahaemolyticus* against Caco-2 cells was estimated 8 h after infection. NE significantly enhanced the cytotoxic activity of *V. parahaemolyticus* against Caco-2 cells (figure 2). The mode of action of NE is generally through interaction with α- and β-adrenergic receptors [20]. Therefore, we examined the relationship of NE and adrenergic receptors on Caco-2 cells with the cytotoxic activity of *V. parahaemolyticus*. Phentolamine (100 μmol/L), a nonselective α-adrenergic antagonist, or propranolol (50 μmol/L), a nonselective β-adrenergic antagonist, reduced the NE-stimulated cytotoxic activity (figure 2) [20]. In the absence of NE, these adrenergic antagonists had no effect on the growth or cytotoxic activity of *V. parahaemolyticus* in DMEM (data not shown). These results indicate that NE stimulates the cytotoxicity of *V. parahaemolyticus* in vitro and that this NE action may be attributed to the adrenergic receptors on Caco-2 cells.

**Effect of NE on the cytotoxicity of TTSS1 and TTSS2 deletion mutant strains.** Recently, it was reported that TTSS1 of *V. parahaemolyticus* was involved in its cytotoxic activity [16]. To analyze the effect of NE on the cytotoxicity related to the TTSSs, we constructed each of the TTSS1 and TTSS2 deletion mutant strains. Infection with the TTSS1 deletion mutant did not cause cytotoxicity irrespective of the presence or absence of NE. In contrast, the cytotoxicity of the TTSS2 deletion mutant was identical to that of the parent strain and was stimulated by NE (figure 3). These results indicate that the enhancement of cytotoxic activity induced by NE depends on the function of TTSS1 but not of TTSS2.
Figure 2. Cytotoxicity of *Vibrio parahaemolyticus* against Caco-2 in the presence of norepinephrine (NE; 50 μmol/L) and adrenergic antagonists. Before use, Caco-2 cells were washed with PBS, and 2 mL of Dulbecco’s modified Eagle medium were added. NE was added to the medium 5 min before bacterial exposure. Antagonists were added to the medium 5 min before NE addition. Caco-2 cells were infected with bacteria (cfu/mL). Eight hours after infection, the cytotoxic activity was assayed by measuring the total cellular lactate dehydrogenase release into the supernatant. Data are values, and statistical significance was set at (asterisks). The results represent 5 independent experiments. Phe, phentolamine (100 μmol/L); Pro, propranolol (50 μmol/L); Vp, *V. parahaemolyticus* strain RIMD2210633.

Stimulation of transcription of virulence-associated genes in the presence of NE. We speculated that NE stimulated the expression of some genes associated with TTSS1, which contributed to the cytotoxicity of *V. parahaemolyticus* in the presence of NE. To test this hypothesis, we performed quantitative real-time RT-PCR for virulence-associated genes [16, 17] (table 1).

Before performing this experiment, we determined to use the 16s rRNA levels as a normalization control for this experiment, because transcription levels of *rrsA* did not vary significantly in DMEM cultures (data not shown). Because host-cell rounding and detachment could be observed 7 h after infection, RNA preparation from infected *V. parahaemolyticus* was performed 6 h after infection, to prevent contamination with components of dead host cell.

When Caco-2 cells were infected with *V. parahaemolyticus* in the presence of NE, the transcription of genes associated with TTSS1 increased, compared with in the absence of NE. In particular, the transcription of *vscQ* and *vscU*, which are TTSS1-related genes, increased by ~3-fold and 5-fold, respectively [16, 21] (figure 4). In addition, the transcription of *vscR*, which is another TTSS1-related gene, also increased somewhat in the presence of NE. In contrast, the transcription of TTSS2-related and neighboring genes did not change in transcription with the addition of NE. Furthermore, the transcription of *tdh*, which is considered to be a major virulence factor in this organism, was unaffected by NE [12, 22–24] (figure 4). These results indicate that NE regulates the transcription of virulence-associated genes related to TTSS1. Therefore, we speculate that the elevation of cytotoxic activity toward Caco-2 cells in the presence of NE could be responsible for the stimulation of *vscQ* and *vscU* gene transcription.

Effect of NE on enterotoxicity in the rat ileal loop model. The ligated ileal loop is recognized as a model for diarrheal disease and represents the human enterotoxicity of pathogenic bacteria [25, 26]. Previous studies have shown that fluid accumulation by *V. parahaemolyticus* in the ligated ileal loop model is exhibited in animal models [16, 27]. Therefore, we examined the effect of NE on the enteropathogenicity of *V. parahaemolyticus* in a rat ligated ileal loop model. Bacterial cultures were supplemented with NE and adrenergic antagonists, and 500 μL was injected into each of the ligated ileal loops. NE at a concentration of 50 μmol/L had no effect on the enterotoxicity, irrespective of the number of injected bacterial cells (data not shown). However, in the presence of 100 μmol/L NE, fluid accumulation increased significantly (ratio, 0.295 ± 0.03 mL/cm), compared with that in the absence of NE (ratio, 0.156 ± 0.04 mL/cm). PBS containing 100 μmol/L NE alone resulted in very little fluid accumulation (ratio, 0.021 ± 0.013 mL/cm), indicating the NE alone, without bacteria, did not induce fluid accumulation in the rat ileal loop model (figure 5).
Adrenergic antagonists were used to analyze whether the enteropathogenicity of *V. parahaemolyticus* was mediated via adrenergic receptors. The nonselective α-adrenergic antagonist phentolamine (100 μmol/L) prevented fluid accumulation, whereas the nonselective β-adrenergic antagonist propranolol (100 μmol/L) had no effect on fluid accumulation (ratios, 0.144 ± 0.043 and 0.254 ± 0.052 mL/cm, respectively). Furthermore, NE-stimulated fluid accumulation was inhibited by prazosin (10 μmol/L), an α1-adrenergic antagonist, but not by yohimbine (100 μmol/L), an α2-adrenergic antagonist (ratios, 0.108 ± 0.036 and 0.205 ± 0.039 mL/cm, respectively) (figure 5) [20]. These results strongly suggest that the effect of NE on the enteropathogenicity of *V. parahaemolyticus* involves the α1-adrenergic receptors on intestinal epithelial cells but not the α2- or β-adrenergic receptors.

**Effect of NE on the enteropathogenicity of TTSS1 and TTSS2 deletion mutants.** It has been reported that TTSS2 of *V. parahaemolyticus* is involved in enteropathogenicity [16]. Therefore, we used TTSS1 and TTSS2 deletion mutant strains to investigate the effects of NE on TTSSs in the rat ileal loop model. The fluid accumulation with the TTSS2 deletion mutant was small (ratios, 0.069 ± 0.016 mL/cm for the presence of NE and 0.063 ± 0.017 mL/cm for the absence of NE). In contrast to the TTSS2 deletion mutant, the enteropathogenicity of the TTSS1 deletion mutant was similar to that of the parent strain (ratios, 0.184 ± 0.031 mL/cm for the TTSS1 deletion mutant and 0.154 ± 0.040 mL/cm for the parental strain), in agreement with a previous study [16]. NE enhanced the fluid accumulation of the TTSS1 deletion mutant, as well as that of the parental strain (ratios, 0.287 ± 0.033 mL/cm for the TTSS1 deletion mutant and 0.295 ± 0.029 mL/cm for the parental strain) (figure 6). These results indicate that NE stimulates the enteropathogenicity of the TTSS1 deletion mutant but not of the TTSS2 deletion mutant.

**DISCUSSION**

*V. parahaemolyticus* displays cytotoxicity toward eukaryotic cells and enterotoxicity in the animal ligated ileal loop model [16, 27–29]. The identification of the virulence determinants of this organism should reveal how the clinical manifestations of *V. parahaemolyticus* infection are generated.

In the present study, we have shown that the pathogenicity on cytotoxic activity of *V. parahaemolyticus* is stimulated in the presence of NE (figure 2). A previous study has demonstrated that TTSS1 is involved in the cytotoxicity of *V. parahaemolyticus* toward a mammalian cultured cell line [16]. The results of our quantitative real-time RT-PCR demonstrate that the transcription of 3 genes related to TTSS1 (vscQ, vscR, and vscU) increases in the presence of NE under infectious conditions (figure 4). To generate cytotoxicity toward host cells via the TTSS, pathogens have to secrete and translocate virulence factor proteins.

![Figure 4](image_url)  
**Figure 4.** Increases in *Vibrio parahaemolyticus* virulence–associated gene transcription in the presence of norepinephrine (NE). Fold change in gene transcription in *V. parahaemolyticus* grown in Dulbecco’s modified Eagle medium with 50 μmol/L NE 6 h after infection of Caco-2 cells, as measured by quantitative real-time polymerase chain reaction. Data analysis was as in Leverton and Kaper [17]. Data are mean ± SE values, and statistical significance was set at *P* < 0.01 (asterisks). The results represent 5 independent experiments. TTSS, type III secretion system.

![Figure 5](image_url)  
**Figure 5.** Fluid accumulation in the rat ileal loop model in the presence of norepinephrine (NE; 100 μmol/L) and adrenergic antagonists. Fluid accumulation is the amount of accumulated fluid (in milliliters) per length (in centimeters) of ligated rat small intestine. The results are mean ± SE values in 12 experimental animals. Statistical significance was set at *P* < 0.01 (asterisks). Ph, phentolamine (100 μmol/L); P, prazosin (10 μmol/L); Pro, propranolol (100 μmol/L); Yoh, yohimbine (100 μmol/L).
through the TTSS [15]. Although it has been speculated that all 3 of these gene products are translocation proteins of TTSS1 [21], the functions of these proteins are still unknown. However, when we performed the infection assay with vscQ and vscU deletion mutant strains, we found that both genes were important for the cytotoxic activity of V. parahaemolyticus (data not shown). Therefore, we speculate that NE may enhance the transcription of several TTSS1 genes, including vscQ and vscU, leading to elevated cytotoxic activity. However, we do not know how NE regulates the transcription of TTSS1 genes in V. parahaemolyticus. Recently, it has been reported that NE might affect the quorum sensing system of EHEC [30]. Quorum sensing is considered to be a cell-to-cell communication system in many bacteria and regulates TTSS1 in V. parahaemolyticus [21, 31]. Thus, we hypothesize that NE may regulate the transcription of TTSS1-related genes through a quorum sensing system. In future studies, it will be of interest to investigate the potential detailed mechanisms of NE stimulation of TTSS1-related gene transcription.

It is well known that the effects on NE are dependent on α- and β-adrenergic receptors on mammalian cells [20]. However, there is no protein in V. parahaemolyticus or other gram-negative bacteria with homology to α- and β-adrenergic receptors [14, 32]. Therefore, it is hard to think that the α- and β-adrenergic antagonists affect V. parahaemolyticus directly. Thus, we speculate that α- and β-adrenergic antagonists may affect the host cell, rather than the bacteria, through interactions with α- and β-adrenergic receptors. Thus, we suggest that the elevation of V. parahaemolyticus cytotoxic activity of against Caco-2 cells is attributable to the dual modulations of NE through interactions with adrenergic receptors on Caco-2 cells and regulations of gene expression in the bacteria. Previous studies have shown that the adrenergic receptors on Caco-2 cells do not include α₁- and β₁-adrenergic receptors [33–35]. Although we performed the infection assay using the α₁-adrenergic antagonist prazosin and the β₁-adrenergic antagonist atenolol to confirm the interaction of each adrenergic receptor on Caco-2 cells with V. parahaemolyticus cytotoxicity [20], neither antagonist inhibited the cytotoxic activity of V. parahaemolyticus (data not shown). It seems likely that both antagonists had toxic effects, because the Caco-2 cells were exposed to each antagonist for a long time (∼8 h). We therefore could not examine the mechanisms by which the adrenergic receptors contributed to the cytotoxicity. However, we estimate that the effect of NE on V. parahaemolyticus cytotoxic activity against Caco-2 cells may be partly associated with both adrenergic receptors.

We also have shown that NE appears to increase V. parahaemolyticus fluid accumulation in the rat ileal loop through interactions with α₁-adrenergic receptors (figure 6). A previous study has shown that NE increases active chloride secretion through interactions with α₁-adrenergic receptors in the porcine distal colon [36]. Furthermore, α₁-adrenergic receptors exist on intestinal epithelial cells, and chloride secretion seems to be an important component of intestinal fluid in diarrhea [35–40]. We think that the elevation of fluid accumulation by NE is primarily attributable to interactions with α₁-adrenergic receptors but not to the stimulation of transcription of TTSS2-associated genes because NE has little effect on their transcription (figure 4). We have no information as to whether NE regulates the transcription of TTSS2-associated genes under in vivo conditions such as in the rat ileal loop model. Although we attempted to perform quantitative real-time RT-PCR in the rat ileal loop model to address this question, we did not obtain good results because we do not know which V. parahaemolyticus genes are suitable for normalizing the experimental results. Thus, we speculate that α₁-adrenergic receptors in the small intestine could be important for the diarrhea caused by V. parahaemolyticus infection.

Whereas a high concentration of NE (100 μmol/L) stimulates enteropathogenicity in the rat ileal loop test (figure 5), an NE effect is not observed at a concentration of 50 μmol/L, the concentration of NE used in the infection assay. Numerous commensal bacteria live on the luminal surface, and many kinds of endocrine hormones exist in the gastrointestinal tract. It is possible that NE injected into the ligated loops is degraded through the activity of host cells or commensal bacteria. Therefore, we postulate that the minimum concentration of NE for the V. parahaemolyticus pathogenicity may be nearly 100 μmol/L in the rat ileal loop model. Although we do not know whether
the concentrations of NE used in this study are related to physiological concentrations, the mesenteric organs produce more than half of all NE in the entire body, and the gastrointestinal epithelium is likely to have higher concentrations of NE than other organs [2]. Furthermore, NE concentrations in the intestinal lumen increase in response to stressors and infectious disease [4, 5]. However, the amount of NE in the intestinal lumen has not been determined, because quantification of NE secreted from the epithelium of the gastrointestinal tract is difficult [41, 42].

The present study demonstrates that the neuroendocrine hormone NE can modulate V. parahaemolyticus pathogenicity via the TTSSs. Many reports on the effects of NE have shown that other pathogenic bacteria also responded by stimulation of growth and pathogenicity [7–9, 43–48]. It seems likely that some types of pathogenic bacteria have conserved strategies for responding to host neuroendocrine hormones. The underlying mechanisms mediating NE and adrenergic receptors that contribute to bacterial pathogenesis remain unclear. Recently, it has been shown that NE stimulates EHEC adherence to porcine intestinal epithelial cells and that this effect seems to be associated with the α2-adrenergic receptors in intestinal epithelial cells [9]. It is possible that detailed analysis of how NE modulates bacterial virulence could lead to the development of new strategies for the prevention and treatment of infectious diseases.

References

26. Twedt RM, Peeler JT, Spaulding PL. Effective ileal loop dose of Kana-


