Inhibition of Small-Intestinal Sugar and Amino Acid Transport by the Enterotoxin of *Shigella dysenteriae* I

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The enterotoxin of *Shigella dysenteriae* I produces fluid and electrolyte secretion in the rabbit ileum. These present studies were designed to evaluate nonelectrolyte transport in rabbit ileal mucosa exposed to *Shigella* enterotoxin. Both 10 mM galactose and 5 mM L-alanine absorptions were significantly impaired in enterotoxin-exposed ileal mucosa compared with control mucosa. L-Alanine influx was not impaired in two other secretory processes: that induced by cholera enterotoxin and hyperosmolarity. These studies provide evidence that both sugar and amino acid absorptions are diminished in the small intestine by the enterotoxin of *S. dysenteriae* I.

Seven bacteria produce exotoxins that stimulate small-intestinal secretion of fluid and electrolytes (1, 2, 6, 9). The enterotoxin of *Vibrio cholerae* has been studied in greatest detail and is responsible for the profuse watery diarrhea of clinical cholera (5, 7). It is well accepted that this enterotoxin activates an active transport process by stimulating mucosal adenylate cyclase. In both clinical and experimental cholera, the small-intestinal mucosa appears normal, and intestinal absorptive function is intact.

In contrast, the enterotoxins produced by *Klebsiella pneumoniae* and *Shigella dysenteriae* I produce mucosal damage, and exposure of jejunal mucosa to *Klebsiella* enterotoxin results in a decrease in sugar absorption (9, 10, 12, 13). We previously provided evidence that the enterotoxin of *S. dysenteriae* I will partially inhibit glucose stimulation of the electrical potential difference (PD) and short-circuit current (Isc), which suggests that glucose stimulation of Na transport is partially inhibited (4). Further, Na transport is diminished across *Shigella* enterotoxin-treated ileal mucosa (4). To date there have been no studies that have directly evaluated nonelectrolyte transport in small intestine exposed to the enterotoxin of *S. dysenteriae* I. These present studies provide evidence that exposure of ileal mucosa of the rabbit to *Shigella* enterotoxin results in an impairment of both sugar and amino acid transport.

**MATERIALS AND METHODS**

Nonfasting New Zealand female rabbits weighing 2 to 3 kg were employed in all experiments. Under pentobarbital anesthesia, loops of ileum approximately 10 cm long were isolated, and 3 ml of a test solution was introduced into the segment. Doubly tied ligatures were placed at each end of the segment; no more than four segments were filled in any animal. A 2-μg portion of *Shigella* enterotoxin in 3 ml of a Ringer solution was placed in experimental loops, and a Ringer solution (3 ml) was used in the control loops. At least one control loop was present in each animal, and if any control segments had more than 3 ml of fluid after the 5-h incubation, none of the segments in that rabbit were used. In another series of experiments, two additional solutions were employed: a cholera solution that contained 5 μg of choleragen, and an hyperosmolar solution of 140 mM NaCl and 100 mM mannitol that had a final osmolarity of approximately 370 mosM/kg of water.

Five hours after the construction of the loops, the animal was sacrificed and the loops of ileum, which had been initially filled with the solutions described above, were removed and emptied. Segments of ileum from these rabbits were then studied by two different techniques. The first method consisted of stripping the serosa and part of the muscular layer from the mucosa, which was then mounted between half Lucite chambers and bathed in a bicarbonate Ringer solution as described previously (17). The PD and Isc were monitored for 40 min, at which time 30 mM 3-O-methyl glucose was added to both the mucosal and serosal solutions. The electrical parameters were then followed for an additional 50 min.

In the other group of experiments, segments of ileum were placed in Lucite influx chambers (16). Four pieces of mucosa from a single segment could be studied simultaneously. During the 40-min preincubation period, the mucosa was bathed in a bicarbonate Ringer solution. The mucosal influx of either 5 mM L-alanine or 10 mM galactose was then measured during a 60-s test period as described previously (16). [3H]Inulin was used to determine the
extracellular fluid space. Uptake was expressed as micromoles per hour per square centimeter. [3H]inulin, L-[3H]alanine, and [14C]galactose were purchased from New England Nuclear Corp. Results are expressed as the mean ± the standard error of the mean. An unpaired Student's t test was employed to determine statistical significance of the results.

RESULTS

During the 5-h incubation period, 10-cm segments of ileum incubated with 3 ml of solution containing Shigella enterotoxin always secreted fluid and electrolytes compared with control segments, which absorbed fluid. Similar to our previous studies (4), PD and Isc were similar across in vitro ileal mucosa exposed to either Shigella enterotoxin or the Ringer solution in vivo. The addition of 30 mM 3-O-methylglucose to both the mucosal and serosal solutions resulted in an abrupt increase in PD (3.4 ± 0.6 mV) and Isc (127.3 ± 11.5 μA/cm²) in control ileum. In contrast, in ileum initially incubated with Shigella enterotoxin, a significantly smaller increment in PD (2.0 ± 0.1 mV, P < 0.05) and Isc (66.6 ± 7.4 μA/cm², P < 0.01) occurred after the addition of 30 mM 3-O-methylglucose. These results, which demonstrate a defect in 3-O-methylglucose stimulation of Isc and PD, are consistent with either a defect in glucose transport or an uncoupling of glucose and Na transport. Therefore, influx studies were performed to measure directly the uptake of a sugar, galactose, and an amino acid, L-alanine.

Galactose influx in 12 control tissues from three animals was 2.91 ± 0.36 μmol/h per cm² and was significantly greater than that observed in ileal mucosa exposed to Shigella enterotoxin (1.43 ± 0.13 μmol/h per cm², P < 0.01). The results of L-alanine influx were similar to those of galactose in that L-alanine influx in Shigella enterotoxin tissue was significantly less than that in control tissues (P < 0.01) (Table 1).

To validate that these results were secondary to the effect of Shigella enterotoxin and not a nonspecific effect of fluid secretion induced by Shigella enterotoxin, L-alanine influx was determined in additional experiments in which ileal mucosa was initially exposed for 5 h to either choleragen or a hyperosmolar solution. L-Alanine influx, in contrast to that observed in ileal mucosa exposed to Shigella enterotoxin (Table 1), was similar to that of control mucosa in these two secretory states. Influx was 4.17 ± 0.50, 4.02 ± 0.23, 3.99 ± 0.47 μmol/h per cm², respectively, in control tissues, tissues exposed to enterotoxin, and tissues exposed to hyperosmolar solution.

<table>
<thead>
<tr>
<th>Table 1. Influx of 10 mM galactose and 10 mM L-alanine in control and Shigella enterotoxin-exposed ileum</th>
<th>Influx</th>
<th>Control</th>
<th>Shigella enterotoxin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Galactose</td>
<td>2.91 ± 0.36</td>
<td>1.43 ± 0.13</td>
<td></td>
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<tr>
<td>(12)</td>
<td>(12)</td>
<td></td>
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<tr>
<td>L-Alanine</td>
<td>4.53 ± 0.39</td>
<td>2.53 ± 0.17</td>
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<td>(16)</td>
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a All results are expressed as the mean ± standard error of the mean in micromoles per hour per square centimeter. Ileal loops were filled with either 2 μg of Shigella enterotoxin or Ringer solution. After 5 h, the ileal segments were removed and placed in Lucite chambers. Galactose and L-alanine influxes were then determined by previously described methods. Numbers in parentheses represent the number of tissues studied. Four pieces of tissue were usually obtained from a single rabbit.

b P < 0.01.

DISCUSSION

These results provide convincing evidence that a defect in both hexose and amino acid transport is present in ileal mucosa exposed in vivo to Shigella enterotoxin. These results are not surprising in view of previous studies of Shigella enterotoxin, which indicated that the enterotoxin produced functional and morphological changes in small intestinal mucosa: significant histological damage was observed with light microscopy, and the increment in PD and Isc after the addition of glucose was impaired (4, 10, 12, 13). Although it is not certain why Shigella enterotoxin possesses these cytotoxic properties, it would appear most reasonable to anticipate that these abnormalities in nonelectrolyte transport are related to this morphological damage. These results provide additional evidence of the cytotoxic effects of Shigella enterotoxin. Keusch and Donta recently provided evidence that the Shigella enterotoxin damages different cells in tissue culture (11).

Neither morphological damage nor functional evidence of a defect in absorption is apparent in ileal mucosa exposed to choleragen enterotoxin. More importantly, this phenomenon of intact absorptive function in cholera enterotoxin-exposed mucosa has been taken advantage of in the treatment of clinical cholera. The ability of "oral therapy" to decrease both stool losses and intravenous fluid requirements is based on the ability of oral glucose to stimulate normal sodium and water absorption and thereby decrease net fluid secretion (8). Escherichia coli enterotoxin also does not alter absorptive function to any significant degree. Klipstein et al. recently provided convincing...
evidence that jejunal mucosa in the rat exposed to an enterotoxin produced by *K. pneumoniae* develop defects in both amino acid and glucose transport (9). Significant histological damage is observed in jejunal and ileal mucosa exposed to *Staphylococcus aureus* enterotoxin, but it is unknown whether a defect in glucose absorption exists in these tissues (15). It was previously emphasized that an impairment in glucose absorption exists in other diarrheal illnesses such as experimental salmonella enterocolitis (14). Therefore, demonstration of a net secretory state cannot be equated with normal absorptive function, and utilization of oral glucose therapy in the treatment of the diarrhea cannot be justified until there is convincing evidence that intestinal absorptive function is not altered in the specific secretory diarrheal illness in question.

Although the enterotoxin of *Shigella dysenteriae* I produces fluid secretion in the ileum of the rabbit, it is still uncertain whether this enterotoxin is directly involved in the natural history of shigellosis. Previous studies indicate that the *Shigella* enterotoxin does not affect colonic fluid electrolyte movement when exposed to the mucosa, but whether colonic secretion occurs when the enterotoxin is elaborated in situ by organisms in the mucosa after invasion is unknown (3).

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