Escherichia coli enterotoxin receptors: localization in opossum kidney, intestine, and testis

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FORTE, LEONARD R., WILLIAM J. KRAUSE, AND RONALD H. FREEMAN. *Escherichia coli enterotoxin receptors: localization in opossum kidney, intestine, and testis.* Am. J. Physiol. 257 (Renal Fluid Electrolyte Physiol. 26): F874–F881, 1989.—The distribution of receptors for *Escherichia coli* enterotoxin were examined in opossum kidney, intestine, and testis. *E. coli* enterotoxin stimulated guanosine 3′,5′-cyclic monophosphate (cGMP) production in renal cortex, testis, and small intestinal mucosa but had only a small effect in the colon. Atrial natriuretic factor enhanced the cGMP content of renal cortex and small intestine but had no effect on testis or colon. The enterotoxin receptors were observed to be localized in proximal tubules, to epithelial cells of crypts and villi of small intestine, to crypts of colon, and in seminiferous tubules. Both convoluted and straight portions of proximal tubules exhibited specific binding sites for ^{125}I-labeled enterotoxin. Glomeruli and distal tubules did not have receptors. Binding of ^{125}I-enterotoxin to brush-border membranes of kidney cortex or intestinal mucosa and to testis membranes was markedly temperature dependent. The binding affinities of these receptors for *E. coli* enterotoxin were similar (i.e., $IC_{50} \cong 0.4$–0.5 nM). Daily administration of 20 μg of enterotoxin intramuscularly to opossums increased urine cGMP excretion with no apparent changes in urine volume, Na^+, or K^+ excretion. Thus receptors for heat-stable enterotoxins are localized to proximal tubules of kidney and to enterocytes and seminiferous tubules of intestine and testis, respectively. Apical membranes may be the site of enterotoxin receptors in these epithelia.

guanosine 3′,5′-cyclic monophosphate; receptor autoradiography; atrial natriuretic factor; brush-border membranes

HEAT-STABLE PEPTIDES belonging to a class of diarrheal enterotoxins are produced by *Escherichia coli*, *Yersinia enterocolitica*, and other pathogenic enteric bacteria (13). Specific, high-affinity binding sites for these peptides are found on the apical membrane of intestinal epithelial cells (8, 9, 12, 14, 17, 23). These enterotoxins activate a membrane-bound form of guanylate cyclase, which leads to an increase in guanosine 3′,5′-cyclic monophosphate (cGMP) content of enterocytes (8–10, 18–20, 28, 29). Analogues of cGMP cause changes in the transport of solute and water in the intestine similar to the effects of bacterial enterotoxins (10, 16, 20, 29). Thus the secretory diarrhea caused by this class of heat-stable (ST) enterotoxins has been postulated to be mediated by the intracellular second messenger, cGMP (10). This cellular mitters, which promote Cl^- secretion in the intestine and in cultured intestinal cell lines via activation of adenylate cyclase (3, 6, 7, 26, 30, 32). *E. coli* enterotoxin also stimulated transepithelial Cl^- secretion in cultured T-84 cells, a human colon carcinoma cell line having receptors for the enterotoxin linked positively to the activation of guanylate cyclase (18, 21).

The biological actions of ST enterotoxins were considered to be restricted to the enterocytes of small or large intestine which expressed apical membrane receptors for these peptides (8, 19, 28). However, we reported recently that opossum kidney (OK), as well as cultured kidney cell lines (PtK-2) had specific, high-affinity binding sites for ^{125}I-labeled enterotoxin (11). Moreover, *E. coli* enterotoxin elicited large increases in kidney or intestinal cGMP production in vitro. Intravenous injection of *E. coli* enterotoxin caused 10- to 50-fold increases in urinary cGMP excretion in this species. Thus renal receptors for the *E. coli* enterotoxin and an associated guanylate cyclase were present in the kidney and these receptors were activated by systemic administration of the enterotoxin. Therefore, ST enterotoxin receptors are more widely distributed in epithelial tissues than was previously considered. In the present study we report that specific receptors for *E. coli* enterotoxin, which are positively coupled to guanylate cyclase, appear to be localized to the proximal tubule in the renal cortex of the North American opossum (*Didelphis virginiana*). Enterotoxin receptors were also localized to the enterocytes of both crypts and villi of small intestine, crypts of large intestine, and to seminiferous tubules of testis. Brush-border membranes prepared from renal cortex or small intestinal mucosa had high-affinity binding sites for ^{125}I-enterotoxin.

EXPERIMENTAL PROCEDURE

Animals. Opossums were trapped locally using Havahart traps (Tomahawk Live Trap, Tomahawk, WI) under a permit from the Missouri Department of Conservation issued to W. J. Krause. Animals of both sexes were housed in the Laboratory Animal Medicine Facility of the School of Medicine. They were fed Purina dog chow (Ralston Purina, St. Louis, MO) and provided with water ad libitum. The animals appeared to be in good health when used in the experiments. Animals were killed by

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