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

LEONARD R. FORTE, WILLIAM J. KRAUSE, AND RONALD H. FREEMAN
Departments of Pharmacology, Anatomy, and Physiology, School of Medicine, University of Missouri, and Harry S. Truman Memorial Veterans Administration Medical Center, Columbia, Missouri 65212

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 mechanism may be analogous to the form of transmembrane signaling observed for hormones and neurotransmitters, 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 an overdose of ketamine administered intracardially followed by exsanguination.

Tissue cGMP measurements. Opossum tissues were dissected free and placed into ice-cold 0.9% NaCl. Tissue slices were prepared with a Stadie-Riggs microtome and placed into Dulbecco's modified Eagle's medium (DMEM) containing 20 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer, pH 7.4 at 4°C until incubated with agonists (11). Tissue slices or intestinal mucosa (usually 125–150 mg wet wt) were incubated for 40 min at 37°C with *E. coli* enterotoxin-STa (Sigma Chemical, St. Louis, MO), atrial natriuretic factor (ANF, Peninsula Laboratories, Belmont, CA), or vehicle that was added to DMEM-HEPES (0.2 ml) plus 1 mM methylisobutylxanthine (MIX). Perchloric acid was then added to a final concentration of 3.3%, slices homogenized, centrifuged, and the supernatants neutralized with 10 N KOH. After centrifugation the supernatant solution was used to measure cGMP by radioimmunoassay (11). Mucosal cGMP content in small intestine and colon was expressed per milligram protein (25).

Iodination of the E. coli enterotoxin. Iodination of *E. coli* enterotoxin with ^{125}I was accomplished by means of lactoperoxidase as described by the manufacturer (Enzymobeads, Bio-Rad Laboratories, Richmond, CA). ^{125}I-enterotoxin was purified by use of reverse-phase high-performance liquid chromatography (HPLC) with a C_{18} column (3.9 mm × 30 cm μBondapak, Waters, Milford, MA). The peak of radioligand used in these experiments was that which corresponded to *peak B* of the HPLC purification method described by Thompson et al. (31). No detectable free ^{125}I was observed in this peak from the linear gradient of acetonitrile. We routinely used the fraction of ^{125}I-enterotoxin that provided the least amount of nonspecific binding in relation to total binding of the radioligand by intestinal brush-border membranes.

Preparation of membranes from kidney, intestine, and testis. Testes from each opossum were homogenized for 35 s in 3 ml/g tissue of a solution containing 0.25 M sucrose, 0.01 M tris(hydroxymethyl)aminomethane (Tris)·HCl, pH 7.4, 1 mM EDTA by means of a Tissumizer (Tekmar, Cincinnati, OH). The homogenate was centrifuged at 43,500 *g* for 15 min and the pellet was resuspended in the same buffer. Membranes were stored frozen at −70°C. Brush-border membranes from opossum kidney cortex and small intestinal mucosa were prepared with the use of the magnesium precipitation technique described by Biber et al. (2). Kidney and intestinal brush-border membranes were stored frozen at −70°C in a solution containing 20% glycerol, 300 mM mannitol, 1 mM $MgCl_2$, 50 mM HEPES-Tris, pH 7.5.

Radioligand binding experiments. Kidney or intestinal brush-border membranes were thawed, sedimented by centrifugation at 30,900 *g* for 30 min, and then resuspended in a buffer containing 50 mM Tris·HCl, pH 7.5, 0.1 mM EDTA, 150 mM NaCl. Kidney (20 μg), intestine (10 μg), or testis (150 μg) membranes were incubated in this buffer containing either 30,000 counts per minute (cpm; for kidney and intestine membranes) or 100,000 cpm (for testis membranes) of ^{125}I-enterotoxin in the presence of a range of concentrations of unlabeled enterotoxin from 10 pM to 1 μM. Incubation times and temperatures are given in the appropriate figure legends. At the end of the incubation, 2 ml of ice-cold calcium, magnesium-free phosphate-buffered saline (PBS) was added to each tube. This suspension was filtered by 25 mm diameter glass microfiber filters (GF/F, Whatman International, Maidstone, England). The filters had been pretreated with 0.1% polyethylenimine (Sigma Chemical) before use. The filters were washed three times with 4 ml each of cold wash solution. Radioactivity was measured by gamma scintillation spectrometry.

In vivo autoradiography of ^{125}I-enterotoxin distribution in kidney. A 150-g male opossum was anesthetized with ketamine and then injected with 30×10^6 cpm of ^{125}I-ST intravenously. Ten minutes later, a time when peak urine cGMP responses to *E. coli* ST were previously observed (9), the animal was exsanguinated and perfused via the ventricle with phosphate-buffered saline (PBS) until all blood was removed from the animal. Kidneys were then frozen in liquid N_2. Frozen sections (14 μm) were cut at −20°C and thaw-mounted on cover slips. After air-drying the sections were placed on X-ray film and exposed for 3 mo.

In vitro autoradiography of ^{125}I-enterotoxin binding to kidney, small intestine, colon, and testis. Opossum tissues were frozen in N_2, and 14-μm sections were cut in a cryostat maintained at −20°C. Sections were thaw-mounted onto gelatin-coated slides and air-dried. Mounted sections were stored at −70°C until used. Each section was incubated with 50 μl of DMEM-2-(*N*-morpholino)ethanesulfonic acid (MES), pH 5.5, containing 0.5% bovine serum albumin (BSA) for 15 min at 37°C. Then 50 μl of DMEM-MES containing 500–2,000 cpm/μl of ^{125}I-enterotoxin ± 1 μM unlabeled enterotoxin and 0.5% BSA were added to measure total and nonspecific binding of this peptide to the tissue sections. The sections were incubated for 15 min at 37°C, washed with a gentle stream of ice-cold PBS, and then washed 3 times in 50 ml of ice-cold PBS for 5 min at each wash. Sections were air-dried and placed into contact with Kodak X-Omat AR X-ray film (Eastman Kodak, Rochester, NY) for detection of radioactivity. The slides were then coated with Kodak NTB-2 emulsion, sealed in light-tight boxes, and stored at 4°C for 3–5 wk until developed.

Chronic treatment of opossums with E. coli enterotoxin. Five opossums raised in our colony (7 mo of age, 1.5–2.0 kg body wt) were placed into metabolism cages for at least 1 wk to allow the animals to become accustomed to the new housing conditions. Then 24-h urine samples were collected for 7–10 days comprising a control period. Each animal received 20 μg of *E. coli* enterotoxin by intramuscular injection per day for three consecutive days. Urine was also collected for several days after treatment was discontinued. Urine cGMP was measured by radioimmunoassay and Na^+ and K^+ by flame photometry.

Statistical analysis. The data were analyzed for differences in tissue cGMP content between control and enterotoxin- or ANF-stimulated values by Student's paired *t* test.

Materials. Tissue culture medium (DMEM) was purchased from GIBCO Laboratories (Grand Island, NY).

Na ^{125}I was supplied by Amersham, 14–17 μCi/μg (Arlington Heights, IL). Other chemicals and reagents were purchased from various suppliers.

RESULTS

Opossum tissues were examined for the presence of *E. coli* enterotoxin-stimulated guanylate cyclase activity by measuring cGMP levels of either tissue slices or intestinal mucosa suspensions exposed to vehicle, enterotoxin, or ANF. Under these conditions *E. coli* enterotoxin elicited a 4.6-fold increase in cGMP content of testis (Table 1). Colon did not respond to the enterotoxin with a significant increase in cGMP, although cGMP levels were generally higher in tissues exposed to that peptide. The effect of *E. coli* enterotoxin on renal cortical and small intestinal mucosa cGMP content (i.e., 13-fold and 10-fold, respectively) was substantially greater than the cGMP response of testis. ANF increased the cGMP content of kidney cortex and intestine, but this peptide had no apparent effect on cGMP content of testis or colon under these conditions.

TABLE 1. *Effect of E. coli enterotoxin and ANF on cGMP levels of opossum kidney, testis, and intestine in vitro*

Tissue	cGMP, fmol/mg		
	Basal	Enterotoxin	ANF
Kidney cortex (11)	34±11	446±128*	56±9*
Testis (6)	17±4	79±20*	30±9
Small intestine (4)	398±77	3,842±1,177*	887±213*
Colon (5)	1,764±914	2,436±1,654	2,183±1,657

Values are means ± SE; number of experiments in parentheses. *E. coli* enterotoxin concentration; kidney and small intestine 0.5 μM; other tissues 1 μM; atrial natriuretic factor (ANF) concentration was 0.1 μM. Guanosine 3′,5′-cyclic monophosphate (cGMP) content is expressed as fmol/mg wet wt for kidney and testis, and fmol/mg protein for small intestine and colon mucosa. * $P < 0.05$ compared with basal, Student's paired *t* test.

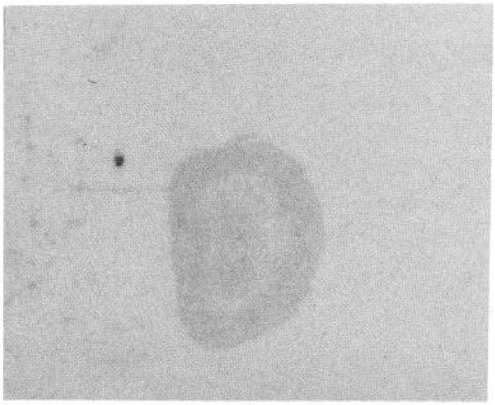

FIG. 1. Localization of ^{125}I-enterotoxin in kidney after in vivo administration of peptide to an opossum. See EXPERIMENTAL PROCEDURE for details of experiment.

In vivo labeling of opossum kidney enterotoxin receptors was accomplished by injecting the ^{125}I-labeled peptide into an opossum. The distribution of radioactivity was highest in renal cortex, much lower in medulla, and intermediate in the renal papilla (Fig. 1). When kidney sections were labeled in vitro with ^{125}I-enterotoxin, we found that specific binding of this radioligand occurred only in the renal cortex (Fig. 2). Radioactivity associated with the medulla or papilla was equivalent to nonspecific binding of the radioligand (denoted by arrows in Fig. 2). In contrast, the testis had a lower apparent binding of ^{125}I-enterotoxin to receptors, which appeared to be distributed evenly throughout the tissue. Small intestine and colon were both labeled by ^{125}I-enterotoxin over the mucosa. We consistently found that brain (cerebral cortex) and skeletal muscle had no specific binding of ^{125}I-enterotoxin (data not shown).

Examination of the ^{125}I-enterotoxin labeled sections of OK by light microscopy revealed that enterotoxin receptors were localized to the proximal tubule (Fig. 3). The pars recta appeared to have a somewhat greater level of radioactivity than did the convoluted portion of the proximal tubule. Glomeruli and distal tubules did not have receptors for this peptide. Also, it appears unlikely that enterotoxin receptors occur in either the loop of Henle or collecting tubules because there was no specific binding of ^{125}I-enterotoxin in the inner medulla or papilla. Enterotoxin receptors in small and large intestine were restricted to the enterocytes of both crypts and villi. No receptors were observed in association with submucosal connective tissue or smooth muscle. The ^{125}I-enter-

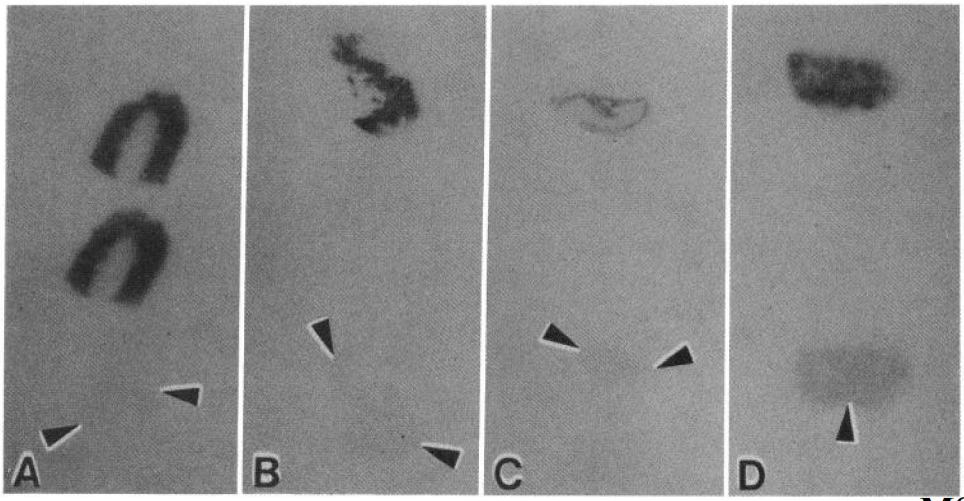

FIG. 2. Specific binding of ^{125}I-enterotoxin to kidney cortex (*A*), small intestine (*B*), and colonic mucosa (*C*) and testes (*D*) revealed by in vitro autoradiography. Data are representative experiments of at least 6 different experiments with frozen sections from these tissues (see EXPERIMENTAL PROCEDURES for details). Exposure of X-ray film varied from 4 to 16 h and data are intended to demonstrate tissue localization and not receptor density. Sections of brain (cortex) and skeletal muscle have consistently shown no specific binding of ^{125}I-enterotoxin (data not shown).

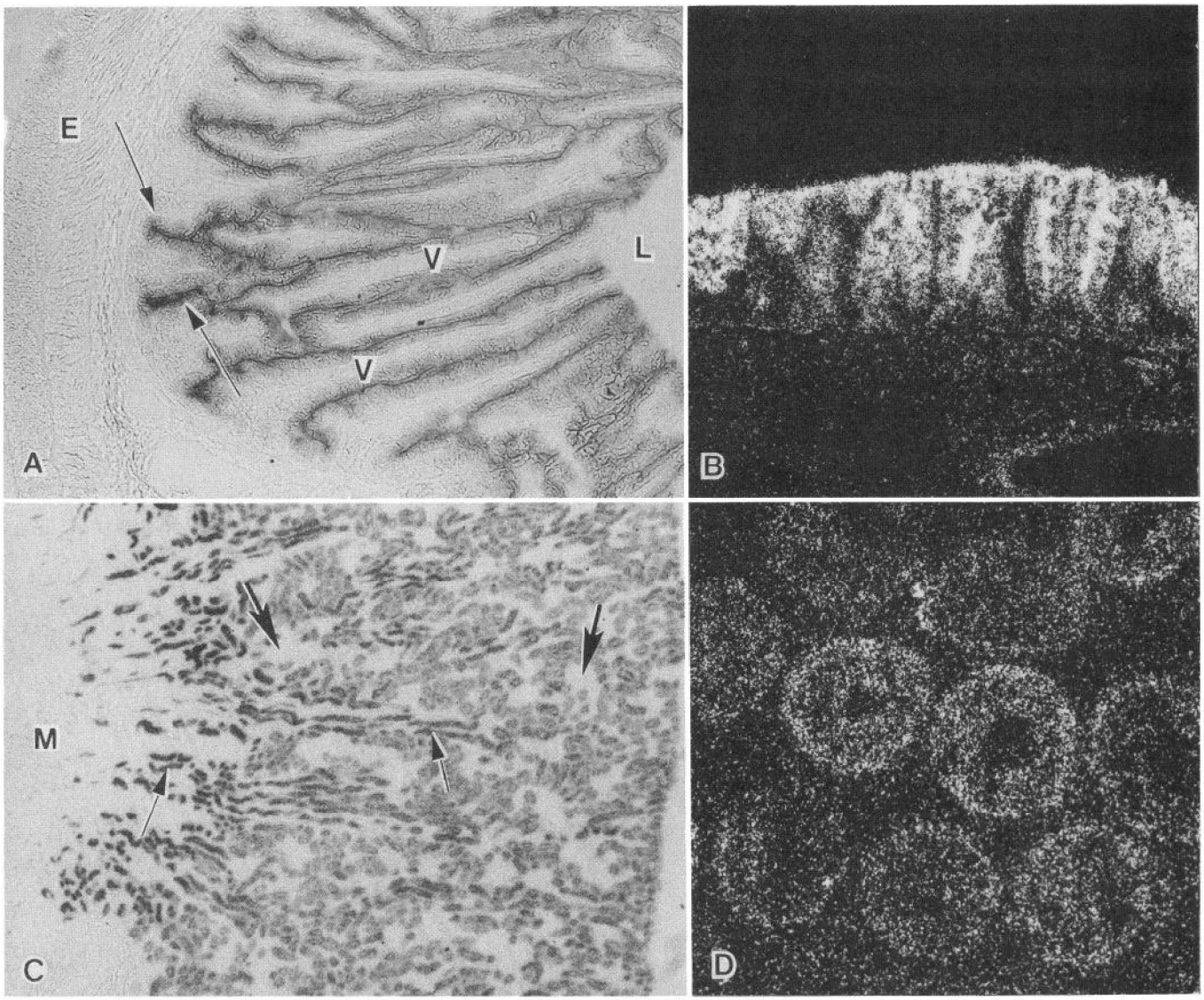

FIG. 3. *A*: radioautograph of a nonstained section from proximal small intestine labeled with ^{125}I-enterotoxin demonstrates receptor localization on intestinal epithelial cells covering villi (v) and lining intestinal glands (arrows). Lumen (L) of intestine is to extreme right. Muscularis externa (E) and connective tissue elements forming submucosa and lamina propria are unlabeled. Magnification ×20. *B*: portion of colon labeled with ^{125}I-enterotoxin and viewed with dark-field microscopy shows intense labeling of intestinal lining epithelium. Magnification ×100. *C*: radioautograph of a nonstained section taken through full thickness of renal cortex and an adjacent portion of medulla shows ^{125}I-enterotoxin labeling of tubules in cortex. Medulla (M) is unlabeled. Tubules concentrated near corticomedullary junction show more intense labeling (small arrows) and these often follow a straight course perpendicular to surface of cortex. Glomeruli (large arrows) are unlabeled. Magnification ×20. *D*: region of testis labeled with ^{125}I-enterotoxin demonstrates receptor localization primarily in seminiferous tubules. Dark field. Magnification ×250.

otoxin receptors were located in the seminiferous tubules of the testis. Thus enterotoxin receptors in these tissues appeared to be expressed only in the epithelial cells of the proximal renal or seminiferous tubules and the intestinal mucosa.

The receptors for ST enterotoxins have been shown to be localized to apical membranes of the intestine (8, 9, 12, 14, 17, 23). Therefore, we prepared brush-border membranes from OK cortex and small intestinal mucosa by a magnesium-precipitation technique (2). Total cellular membranes were prepared from testis. The characteristics of ^{125}I-enterotoxin binding to these preparations were investigated to compare properties of the receptors in these tissues. We found that little specific binding of ^{125}I-enterotoxin to receptors in brush-border membranes from kidney (Fig. 4) or intestine (not shown) occurred at 4°C relative to the binding observed at 37°C. Similar data were obtained with the testis membrane preparation (Fig. 4). The binding of ^{125}I-enterotoxin to kidney, intestinal, and testicular membranes was inhibited by unlabeled peptide between 0.01 and 10 nM of enterotoxin (Fig. 5). The relative affinities of these receptor sites for the *E. coli* enterotoxin were similar under these conditions. The IC_{50} values for *E. coli* enterotoxin inhibition of ^{125}I-enterotoxin binding to kidney, intestine, and testis membranes were 0.5 ± 0.09 (n = 4), 0.4 ± 0.2 (n = 3), and 0.4 ± 0.04 nM (n = 3), respectively.

We previously reported that intravenous administra-

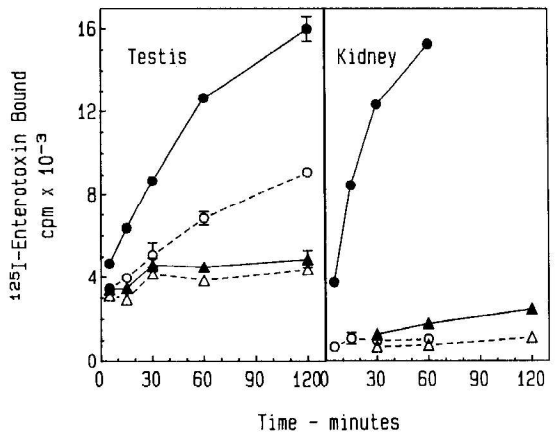

FIG. 4. Time and temperature dependence for binding of ^{125}I enterotoxin to testis and kidney membranes. See EXPERIMENTAL PROCEDURE for methods of membrane preparation. Incubations were carried out at 37°C ●——●, ▲——▲, or 4°C ○——○, △- - - -△. Total binding of ^{125}I-enterotoxin is illustrated by ●——●, ○- - - -○ and nonspecific binding (with 1 μM enterotoxin) by ▲——▲, △- - - -△. Data are means ± SE of triplicates in a representative experiment. Scale on left axis also applies to data in right-hand panel.

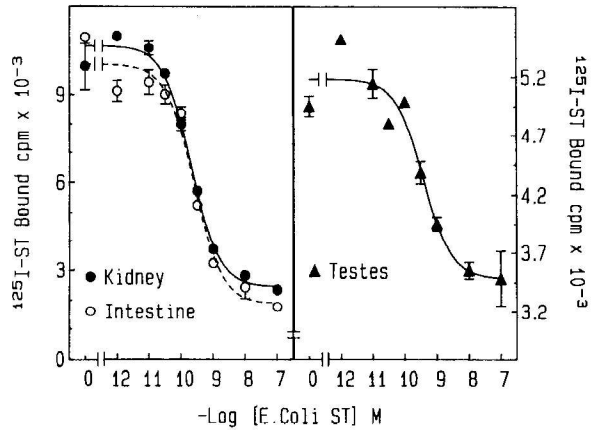

FIG. 5. Comparison of radioligand binding curves for ^{125}I-enterotoxin binding to kidney and intestine brush-border membranes with testis membranes. Data are a representative experiment of a minimum of 3 binding isotherms carried out with each membrane preparation. ST, heat stable.

tion of *E. coli* enterotoxin to the anesthetized opossum resulted in a large increase in urine cGMP excretion without any change in urinary excretion of H_2O, Na^+, K^+, Cl^-, Mg^{2+}, Ca^{2+}, and phosphate (11). In contrast, ANF elicited a smaller increase in cGMP excretion but caused a marked diuresis, natriuresis, and calciuria. In the present study we investigated the effects of chronic administration of *E. coli* enterotoxin on renal function. Intramuscular injection of 20 μg enterotoxin daily for 3 days resulted in a marked increase in urine cGMP excretion in conscious, unrestrained opossums that persisted for 1 day after discontinuation of treatment (Fig. 6). The peptide did not appear to influence urine Na^+, K^+, or H_2O excretion when administered on a daily basis and did not produce diarrhea by this route of administration. Lack of an effect of the enterotoxin on Na^+ and H_2O excretion in this and earlier experiments (11) even though a marked stimulation of cGMP excretion occurred is consistent with the localization of *E. coli* enterotoxin receptors to the proximal tubule. Putative effects

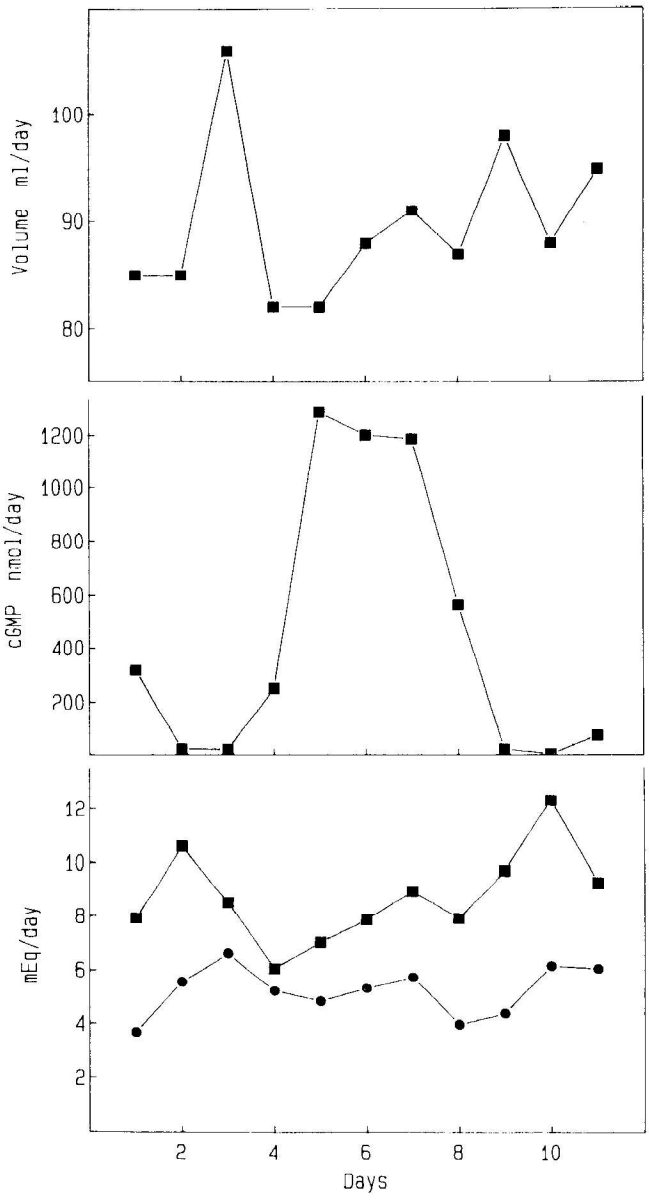

FIG. 6. Chronic administration of *E. coli* enterotoxin to opossum increases urine cGMP excretion. Data are means of 5 animals for each point. Each animal received 20 μg of *E. coli* enterotoxin intramuscularly as a single injection on *days 4, 5,* and *6*. Peak changes in urine cGMP occurred 24 h later at *days 5, 6,* and *7* of urine collection. ■——■ Na^+ excretion, ●——● K^+ excretion.

of this peptide (via cGMP) on ion transport in that nephron segment could be substantially affected by compensatory responses of the nephron segments distal to the proximal tubule.

DISCUSSION

These experiments revealed that renal receptors for *E. coli* enterotoxin are localized to the proximal tubule, presumably in the brush-border membranes of proximal tubular epithelial cells. Moreover, receptor autoradiography revealed that ^{125}I-enterotoxin labeled the receptor sites in epithelial cells of the seminiferous tubules of opossum testis. Enterotoxin receptors were localized to enterocytes of both crypt and villus regions in the small intestine and crypts of the large intestine. Therefore, the

conclusion can be made that the enterotoxin receptor protein may be broadly expressed in several different epithelial cells of the opossum. The enterotoxin stimulates chloride secretion and also inhibits salt absorption in the intestine (6, 10, 13, 16, 29). These effects, which are the apparent cause of "traveler's diarrhea" can be reproduced by cGMP analogues in experimental systems (20). If the apical membrane receptor for ST enterotoxins is broadly expressed in epithelia, perhaps this membrane protein (via cGMP) influences salt transport in kidney, testis, and other epithelia by a mechanism similar to that which regulates salt transport in the small and large intestine.

Data presented in this manuscript suggest that enterotoxin receptors do not occur in glomeruli, distal tubules, thick ascending limb of the loop of Henle, or collecting tubules. The occurrence of high-affinity receptors for ^{125}I-enterotoxin in the proximal tubule (convoluted and pars recta) detected by autoradiography and in brush-border membranes does not rule out the possibility that other nephron segments and basolateral membranes may have this protein. A population of receptors at substantially lower concentration (i.e., density) may not be revealed by the in vitro autoradiography technique used in these experiments. Moreover, we have not measured ^{125}I-enterotoxin binding to putative receptors of kidney basolateral membranes. However, other studies using such approaches with membranes isolated from intestinal mucosa revealed that enterotoxin receptors were localized to the brush-border membranes (4, 8, 9, 12, 17, 23, 28). Thus it may be postulated that this receptor protein, which in the OK cell line was 120 kDa in mass (11), may be an apical membrane protein in proximal tubular cells of OK. A similar size of the enterotoxin receptor of about 100 kDa was observed in the intestine of rats (8).

ST enterotoxins have three physiological actions. The *E. coli* enterotoxin stimulates cGMP production in the intestine (8–10, 18–20, 29), kidney (11), and testis (present study), stimulates chloride secretion in the intestine and in crypt cell-like T-84 cells (13, 21), and inhibits sodium chloride absorption in the intestine (10, 13, 16, 29). Thus "traveler's diarrhea" elicited by heat-stable enterotoxins is a secretory form of diarrhea that is similar to the disorder caused by heat-labile enterotoxins such as cholera toxin (13). Binding of the *E. coli* enterotoxin to apical membranes of T-84 cells caused a marked increase in apical membrane chloride permeability, probably by opening chloride channels in this membrane (21). Because analogues of cGMP mimic the effects of *E. coli* enterotoxin on intestinal ion transport, it has been postulated that cGMP serves as a second messenger for the ST enterotoxins, similar to the adenosine 3′,5′-cyclic monophosphate-mediated effects of cholera toxin to cause secretory diarrhea (10, 13, 16, 20). However, administration of *E. coli* enterotoxin to the opossum acutely (11) or chronically (daily) did not appear to influence urinary Na^+, Cl^-, or water excretion even though large increases in urine cGMP excretion were observed in these experiments. In view of the localization of the enterotoxin receptors to the proximal renal tubule, it may be postulated that the enterotoxin could influence salt transport in this segment in a fashion analogous to the peptides' actions on intestinal salt transport (10, 13, 16, 21, 29). However, nephron segments distal to the proximal tubule may compensate for putative changes in filtrate composition elicited by the enterotoxin's action(s) on proximal epithelial cells. It is of interest that the intestine, unlike the nephron, has receptors for *E. coli* enterotoxin throughout the small and large intestine. Moreover, enterotoxin receptors occurred both in the enterocytes of crypts and villi, which agrees with the cGMP responses to the enterotoxin that were shown in the epithelial cells of crypts or villi (8). Such a distribution of receptors throughout the length of the intestine may contribute substantially to the massive diarrhea produced by ST enterotoxins in mammals (13). The action(s) of *E. coli* enterotoxin on proximal tubular function, if any exist, may require micropuncture and/or perfusion of proximal tubules to assess the effects of this peptide on transport in this nephron segment. Because OK and PtK-2 kidney cell lines express enterotoxin receptors (11), it may be possible to gain new information regarding the physiological actions of ST enterotoxins on renal function by use of these proximal tubulelike cell lines. Because *E. coli* enterotoxin regulates apical chloride permeability in T-84 intestinal cells via control of chloride channels (21), it is tempting to speculate that this peptide could exert a similar influence on apical membrane chloride channels in proximal tubular cells. Such a mechanism implies that the 120-kDa enterotoxin receptor protein (11) may serve as a regulator of apical chloride channels in kidney, intestine, and perhaps other epithelia.

These experiments revealed that a third tissue, testis, expresses receptors for the *E. coli* enterotoxin. These receptors appear to be, like those of OK and intestine, coupled positively to the activation of an apical membrane guanylate cyclase. Membrane-bound guanylate cyclase (22) is also postulated to be an effector mechanism for ANF, because atrial peptides stimulate cGMP production in a number of tissues, including kidney, intestine, and testis (1, 22). We found that ANF stimulated cGMP production in the small intestine and kidney cortex, but ANF was ineffective under these conditions in the testis and colon mucosa of opossums. Thus the relative distribution of the ANF and enterotoxin receptors that are coupled to guanylate cyclase appears to be different in these opossum tissues. Receptors for ANF that may be responsible for at least part of the natriuretic action of this peptide are found in cells of the inner medullary collecting ducts (24, 33). If ANF receptors exist in the terminal portion of the collecting tubule in the opossum, a nephron segment with no apparent enterotoxin receptors, then these receptors may contribute substantially to the natriuresis and diuresis elicited by ANF in vivo (11). Regulation of Na^+ channel permeability of collecting tubules by a cGMP-mediated process has been invoked as a cellular mechanism of ANF action (15, 24, 33). Both OK proximal tubules and the proximal tubular-like (5) OK cell line have enterotoxin receptors coupled positively to guanylate cyclase (11). OK cells also have ANF receptors (27). Thus a useful approach

for future research may be exploring the effects of *E. coli* enterotoxin and ANF on Na^+ permeability by use of the OK cell line as an experimental model for proximal tubular cells of the opossum.

In conclusion, receptors for *E. coli* enterotoxin are localized to epithelial cells of the proximal renal tubule, seminiferous tubules of testis, and enterocytes in both crypts and villi of the small intestine and crypts of the colon. These receptors appeared to be associated with apical membranes of both kidney cortex and small intestine of the opossum. The kidney and testis receptors, similar to the intestine, are coupled to activation of guanylate cyclase. Occurrence of receptors for this family of peptides secreted by pathogenic enteric bacteria in tissues other than the intestine suggests that this 120-kDa membrane protein (11) may have a more general role in epithelial cell function than was previously considered.

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Address for reprint requests: L. R. Forte, Dept. of Pharmacology, School of Medicine, University of Missouri, Columbia, MO 65212.

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