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Effect of intravenous injection of crude cholera enterotoxin on canine small bowel

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In memoriam Oscar Felsenfeld

Summary

Injection of a crude preparation of cholera enterotoxin into the superior mesenteric artery caused isotonic fluid secretion by the canine small bowel. In dogs previously exposed to cellular antigens of *Vibrio cholerae*, the time course of the intestinal response to intra-arterial injection of crude enterotoxin was similar to that observed after intraluminal enterotoxin challenge.

Key words: Vibrio cholerae; cholera enterotoxin; intestinal secretion.

Introduction

Vibrio cholerae elaborates an enterotoxin which, when exposed to the mammalian small bowel, causes prolonged secretion of an isotonic fluid with a characteristic electrolyte pattern (Pierce et al., 1971). The small bowel secretion reaches a maximal rate some 3 to 4 h after exposure of the bowel to the cholera enterotoxin, and then continues at maximum levels for an additional 8 to 10 h (Carpenter and Greenough, 1968). The enterotoxin rapidly binds to the intestinal mucosa, and activates intestinal mucosal adenyl cyclase (Kimberg et al., 1971; Sharp and Hynie, 1971). There is a resultant increase in intracellular cyclic 3′5′ adenosine monophosphate which leads to intestinal fluid secretion (Kimberg et al., 1971; Schafer et al., 1970). In clinical and experimental cholera, there is no evidence that the enterotoxin ever passes beyond the small bowel mucosa, and there are therefore no systemic effects of the enterotoxin (Pierce et al., 1972). Parenteral injection of purified enterotoxin, however, causes widespread systemic effects, presumably mediated by adenyl cyclase stimulation in a variety of cells (Pierce et al., 1972). Intravenous injection of toxin, at doses

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tolerable to experimental animals, has, however, not caused intestinal fluid secretion.

The current studies were carried out in order to determine whether or not exposure of the small bowel to a high concentration of enterotoxin via the intra-arterial route would cause intestinal secretion.

Methods

In a preliminary study, a single healthy 15 kg mongrel dog was used. This animal had previously been challenged, by the orogastric route, with 10^{11} microorganisms of a non-toxigenic strain of *V. cholerae;* there was no detectable intestinal fluid loss in response to this challenge. Following pentobarbital anaesthesia and laparotomy under sterile conditions, 4 g of crude cholera enterotoxin (NIH lot 001) in 10 ml of sterile distilled water was injected in the superior mesenteric artery, over a 2-min period, via a #22 needle. The abdominal incision was then closed, and intestinal fluid output was observed over the following 18-h period via a Foley catheter inserted into the rectum. Intestinal fluid losses were replaced intravenously by equal volumes of lactated Ringer's solution, and the dog was returned to his cage in good condition following the experiment.

Experiment I (6 dogs): Following this demonstration that arterial injection of crude enterotoxin could cause intestinal fluid loss, acute studies were performed in six mongrel dogs weighing from 12 to 18 kg. Each of these dogs was anesthetized with pentobarbital, laparotomy was performed, and the small bowel was divided into three segments. The most proximal, or duodenal, segment extended from the pylorus to the ligament of Treitz. The second, or jejunal, segment of small bowel extended from the ligament of Treitz to a point 70 cm beyond the ligament of Treitz. The third, or ileal segment, included the remainder of the small bowel and terminated at the ileo-cecal junction. Both ends of each of these segments were cannulated by Foley catheters, which were sutured in place with balloons inflated. The two catheters from each segment of small bowel were allowed to drain freely into collecting flasks throughout the experiment. Each segment was flushed with 200 ml of air at hourly intervals throughout the study, and the volume of fluid produced by each small bowel segment was measured at the end of each hourly collection. Fluid obtained from each segment 4 h after the enterotoxin injection was analyzed for sodium, chloride, potassium, carbon dioxide combining power and protein concentrations. Sodium and potassium were determined by flame photometry, chloride was measured by a Cotlov chloridometer, the CO_2 combining power was determined by a Natelson microgasometer, and total protein was measured by the Folin-Ciocalteu method (Lowry et al., 1951). At the end of the fourth hourly collection period, a full-thickness intestinal biopsy was obtained from the midsection of each of the small intestinal segments in five animals; the biopsy specimens were placed in 10% formalin and were later examined histologically.

One hour after completion of the small bowel surgery, each animal received an injection of 8 g of crude cholera enterotoxin (Wyeth lot 001) via the superior mesenteric artery. The enterotoxin was dissolved in 10 ml of sterile distilled water and injected into the superior mesenteric artery through a #22 needle over a 2-min period. The surgical incision was then closed with surgical clamps, and the intestinal fluid was collected over the ensuing 8-h period. Throughout the studies, the hematocrit was maintained at control levels by infusion of lactated Ringer's solution in quantities equal to the intestinal fluid losses.

Experiment II (3 dogs): Identical studies were carried out in three dogs which differed from the dogs in the preceding experiment in that each of these dogs had previously been challenged by orogastric inoculation with 10^{11} viable microorganisms of a nontoxigenic strain of *V. cholerae.* None of these animals had shown a detectable physiologic response to the challenge with the nontoxigenic

Table 1. Hourly isotonic fluid output (ml/cm/h) by small bowel following intra-arterial challenge with crude cholera enterotoxin (mean values ± S.E.M.)

Hour	1	2	3	4	5	6	7
Duodenum	2.0 ± 0.3	2.4 ± 0.3	1.5 ± 0.2	1.2 ± 0.2	1.6 ± 0.2	1.5 ± 0.2	1.3 ± 0.1
Jejunum	0.7 ± 0.3	0.8 ± 0.1	0.8 ± 0.2	0.7 ± 0.2	0.6 ± 0.2	0.7 ± 0.1	0.7 ± 0.2
Ileum	0.9 ± 0.4	1.0 ± 0.2	1.1 ± 0.1	0.9 ± 0.2	1.3 ± 0.3	1.1 ± 0.2	0.9 ± 0.1

Table 2. Electrolyte pattern of fluid secreted by small bowel segments, following intra-arterial challenge with cholera enterotoxin, in animals with no previous exposure to *V. cholerae* (mean values ± S.D., in mEq/l)

	Sodium	Chloride	Potassium	Bicarbonate
Duodenum	142 ± 6	122 ± 8	6.0 ± 2.1	17 ± 8
Jejunum	138 ± 4	135 ± 9	6.2 ± 0.7	10 ± 7
Ileum	137 ± 5	89 ± 18	8.1 ± 2.5	52 ± 13

strain of *V. cholerae*, but each had presumably developed antigens against the cell wall of *V. cholerae* (Sack and Carpenter, 1969).

Experiment III (4 dogs): In addition, studies were carried out on four mongrel dogs, which had no previous exposure either to *V. cholerae* or to the cholera enterotoxin. Each of these animals received, via the superior mesenteric artery, an injection of crude cholera enterotoxin (Wyeth lot 001) which had been inactivated by heating to 80° C for 30 min. The remainder of the protocol in these animals were identical to that in experiments I and II.

Results

In the preliminary study, no fluid output was observed during the first two hours after arterial challenge. Intestinal fluid output began during the third hour after challenge, and during the third through the tenth hours post-challenge 2700 ml of fluid (18% of body weight) was produced via the rectal catheter. Fluid output decreased gradually thereafter, and ceased entirely 18 h after challenge.

In Experiment I, all animals responded to the intra-arterial challenge with crude cholera enterotoxin by a brisk outpouring of isotonic fluids from all segments of the small bowel. In each animal, fluid output began shortly after the crude enterotoxin injection, and the rate of isotonic fluid production reached maximal levels during the first hour after the toxin administration. The fluid output continued at near maximal levels throughout the period of observation. The fluid output, per unit length of small bowel, was greater in the duodenum than in the jejunum and ileum (Table 1). The fluid output per unit length of small bowel was strikingly similar to that observed after intraluminal administration of cholera enterotoxin (Carpenter et al., 1968). Electrolyte pattern of the

Table 3. Hourly isotonic fluid output by small bowel segments, following intra-arterial challenge with crude cholera enterotoxin, in animals previously exposed to nontoxigenic *V. cholerae* (mean values, ml/cm/h)

Hour	1	2	3	4	5	6	7	8
Duodenum	0	0	0.7	0.8	1.2	1.5	1.4	1.1
Jejunum	0	0	0	0.3	0.5	0.8	0.8	0.8
Ileum	0	0	0	0.2	0.5	0.7	0.8	0.5

small bowel fluid (Table 2) was the same as that observed after intraluminal administration of cholera enterotoxin to the canine small bowel (Carpenter et al., 1968). The mean protein concentration of the small bowel fluid was 399 mg%, which was slightly, but not significantly, greater than that observed after intraluminal administration of cholera enterotoxin. Biopsies of small bowel showed no disruption of the mucosal architecture, no damage to the epithelial cell structure and no edema of the lamina propria. Inflammatory cells were seen in the lamina propria in three of the five biopsies, but these were not associated with any evidence of epithelial cell damage.

In Experiment II, no fluid output occurred from the duodenal loop until the third hour after the enterotoxin injection, and no fluid was produced by the jejunal and ileal loops until the fourth hour after enterotoxin injection. Fluid output reached maximal levels during the fifth and sixth hour after the enterotoxin injection, and remained at these levels through the eighth hour of observation (Table 3). Electrolyte patterns and protein concentrations for the respective segments of small bowel were similar to those observed in Experiment I.

In Experiment III, all animals exhibited fluid production, at rates comparable to those observed in Experiment I, during the first three hours after the administration of the enterotoxin. The rate of fluid loss decreased significantly during the fourth hour after the enterotoxin administration, and ceased entirely by the fifth hour after the arterial challenge. Characteristics of the fluid producted in Experiment III were essentially the same as those in I and II.

Discussion

After intraluminal challenge with cholera enterotoxin, there is a characteristic and consistent delay before net fluid secretion occurs in the mammalian small bowel. The cholera enterotoxin acts via stimulation of adenyl cyclase in the intestinal epithelial cells. The resultant accumulation of intracellular cyclic AMP leads to secretion of isotonic fluid by all segments of the small bowel (Pierce et al., 1971). The enterotoxin initially rapidly binds to the gut epithelial cells, and once it has been bound to epithelial cells, adenyl cyclase activation and intestinal fluid secretion occur and persist for many hours. In neither clinical nor experimental cholera is there any evidence that the biologically active

enterotoxin passes beyond the epithelial cell border. The delay of 2 to 3 h before the enterotoxin exerts a maximum effect is observed in other tissues as well, including the rat lipocyte, the human platelet, the canine thyroid and the human leukocyte (Carpenter, 1972). In each of these cells the cholera enterotoxin stimulates adenyl cyclase and the resultant intracellular cyclic AMP accumulation leads to the characteristic effect. A more rapid onset of action of cholera toxin has been demonstrated in broken cell systems (homogenized human platelets and sonicated rat liver cells) in which an adenyl cyclase-mediated effect on glycogenolysis can be demonstrated almost immediately after addition of the enterotoxin (Zieve et al., 1971).

The current studies demonstrate two unexpected characteristics of the crude enterotoxin preparation. First, the crude enterotoxin does cause fluid secretion when introduced by the arterial route, and secondly, the fluid secretion in response to intra-arterial enterotoxin administration in previously unchallenged animals occurs almost immediately.

The first of these findings, that stimulation of fluid secretion occurs when toxin is injected by the arterial route, suggests that active enterotoxin may be able to bind to sites other than the luminal border of the intestinal epithelial cell. In these studies, of course, a very artificial situation prevailed, in that a very large bolus of crude toxin was rapidly injected into the mesenteric circulation, and the concentration of toxin reaching the capillary bed was far greater than that which occurred in earlier studies of the effects of the enterotoxin administered by the intravenous route.

The studies in Experiment II, as well as in the preliminary study, suggest that the moiety in the crude enterotoxin preparation which caused *immediate* fluid secretion is different from the enterotoxin per se. The dogs employed in Experiment II had been previously challenged with a nontoxigenic strain of *V. cholerae*, and presumably had developed antibodies against the cellular antigens of *V. cholerae*, but not against the enterotoxin (Sack and Carpenter, 1969). In these animals, the absence of any outpouring of fluid during the first three hours after the crude toxin was injected, suggests that the animals were protected against whatever toxic moiety was responsible for the initial outpouring of fluid. The animals were not protected from the enterotoxin, which caused the characteristic delayed output of fluid by the fourth hour after the crude enterotoxin had been administered.

The studies in Experiment III, demonstrating that a heat-inactivated toxin preparation caused an early, but not sustained, intestinal fluid output, also support the concept that a heat-stable factor (or factors), other than enterotoxin, was responsible for the immediate outpouring of fluid after administration of the crude enterotoxin preparation. Presumably this heat-stable factor is a cellular antigen which was neutralized by the circulating antibodies present in the animals in Experiment II.

These data indicate that crude cholera enterotoxin, in high dosage, can

cause isotonic fluid secretion by the small bowel when administered via the arterial route. The nature and location of the binding site of the enterotoxin, when administered by this route, is not known. The data also suggest that antigenic heat-stable moieties, present in the crude toxin preparation, may cause an immediate and short-lived intestinal fluid secretion when administered by the arterial route. The latter observation is in no way relevant to clinical cholera, but may be of importance in the further study of intestinal secretory mechanisms.

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