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# Comparison of the Mechanisms of Action of Cholera Toxin and the Heat-Stable Enterotoxins of *Escherichia coli*

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**The mechanisms which enable cholera toxin (CT) and the *Escherichia coli* heat-stable enterotoxins (STa and STb) to stimulate intestinal secretion of water and electrolytes are only partially understood. CT evokes the synthesis of 3′,5′-cyclic AMP (cAMP), and STa is known to elevate intestinal levels of 3′,5′-cyclic GMP (cGMP). Neither of these recognized second messengers appears to mediate *E. coli* STb responses. We compared the secretory effects of CT, STa, and STb using the pig intestinal loop model and also measured the effects of toxin challenge on the synthesis of cAMP, cGMP, and prostaglandins (e.g., prostaglandin $E_2$ [$PGE_2$]), as well as on the release of 5-hydroxytryptamine (5-HT) from intestinal enterochromaffin cells. All three enterotoxins elicited fluid accumulation within a 2-h observation period. A combination of maximal doses of STa with STb yielded additive effects on fluid accumulation, which suggested different mechanisms of action for these toxins. Similarly, challenge of pig intestinal loops with a combination of CT and STb resulted in additive effects on fluid accumulation and luminal release of 5-HT. Unlike its effect on intestinal tissues from other animals, CT did not appear to elicit a dose-dependent cAMP response measurable in mucosal extracts from pig small intestine. In contrast, luminal fluid from CT-challenged pig intestinal loops contained dose-related amounts of cAMP and $PGE_2$ that had been secreted from the mucosa. cAMP responses to STa or STb could not be demonstrated in either mucosal tissue or luminal fluid. In contrast, cGMP levels were increased in the intestinal fluid of loops challenged with STa but not in those challenged with STb. While the mechanisms of action of CT and STa are thought to involve impulse transmission via the enteric nervous system, we demonstrated significant stimulation of $PGE_2$ synthesis and 5-HT release for CT and STb but very little for STa. We conclude from these data that the mechanisms of action of STa, STb, and CT are distinct, although the mode of action of STb may have some similarity to that of CT. Since STb stimulated the release of both $PGE_2$ and 5-HT from the intestinal mucosa, the data suggested the potential for an effect of STb on the enteric nervous system.**

The mechanisms of action of cholera toxin (CT) and the *Escherichia coli* heat-labile enterotoxin are believed to involve stimulation of adenylate cyclase via ADP-ribosylation of the $G_{S\alpha}$ regulatory protein, which increases intracellular 3′,5′-cyclic AMP (cAMP) levels. While this second messenger has the potential to stimulate ion transport channels directly (10), it also causes release of 5-hydroxytryptamine (5-HT) from intestinal enterochromaffin (EC) cells located in the epithelial monolayer (19). Release of 5-HT from the base of the EC cells is thought to initiate signals carried by the enteric neurons to the crypt epithelial cells, resulting in increased $Cl^-$ transport (7, 9, 19), and to circular smooth muscle, resulting in contractile responses (16, 17). In addition, CT is known to elicit synthesis and release of various prostaglandins (21), which have the potential to stimulate electrolyte transport (24) and intestinal motility (16). Further, nonsteroidal anti-inflammatory drugs diminish CT-induced secretion (26).

The pathogenic mechanisms of the heat-stable enterotoxins of *E. coli* (e.g., STa and STb) are less well understood. STa has been reported to stimulate guanylate cyclase (12), and drugs known to block cyclooxygenase inhibit STa-mediated fluid secretion (13). The latter finding suggests some prostaglandin component in the mechanism. Blockade of enteric nerves with drugs (e.g., lidocaine) inhibits the secretory effects of both STa and CT (8, 9). In contrast, the mechanism of action of STb is unknown, but attempts to measure cAMP and 3′,5′-cyclic GMP (cGMP) (15) have revealed no alterations. Further, Weikel et al. (27) reported that STb exerted a unique effect on ion transport thought to involve bicarbonate rather than chloride ions.

Comparative studies of bacterial enterotoxins have been limited by the availability of toxin receptors in the intestinal tracts of experimental animals used to assess their biological activities. The ubiquity of the CT and heat-labile enterotoxin receptor, $G_{M1}$ ganglioside, enables CT and *E. coli* heat-labile enterotoxin to be measured in a variety of tissue culture cell lines and animal models in which intestinal loops are constructed (18). In contrast, study of the biological activity of STa and STb is limited to the infant mouse model or to the rat and pig intestinal loop models (28). The pig loop model is relatively expensive for routine use; however, it offers the advantage that the mechanisms of action of several bacterial enterotoxins can be evaluated simultaneously. In previous studies, CT was observed to elicit fluid accumulation in pig intestinal loops in the absence of a demonstrable increase in mucosal cAMP concentration (11, 14). This unique observation was of particular interest to us during the performance of these experiments. In the present study, we used the pig model to compare the effects of CT, STa, and STb on intestinal fluid accumulation and to measure the mediator responses (e.g., cAMP, cGMP, prosta-

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glandin $E_2$ [$PGE_2$], and 5-HT) as a means of studying the mechanism of action of each toxin.

## MATERIALS AND METHODS

**Reagents.** CT was purchased from List Biological Laboratories (Campbell, Calif.). Purified STa (1) was generously supplied by Don Robertson (University of Idaho, Moscow). STb was purified by the method of Dreyfus et al. (6). Soybean trypsin inhibitor was purchased from Sigma Chemical Co. (St. Louis, Mo.). Butorphanol tartrate (Torbugesic) and a 26% solution of sodium pentobarbitol (Sleepaway) were purchased from Fort Dodge Laboratories (Fort Dodge, Iowa).

**Pig intestinal loop model.** Mixed-breed, 6- to 8-week-old pigs were deprived of food overnight before anesthetization with halothane. Following laparotomy, the lumen of the small intestine was lavaged twice with approximately 50 ml of warm saline, and a series of eight 6-cm loops separated by 3- to 4-cm interloops were constructed as described elsewhere (28). The most proximal loop was placed approximately 100 cm distal to the ligament of Treitz, and 5 ml of each randomly assigned test material was injected into the intestinal lumen with 26-gauge needles. To minimize enzymatic degradation of the enterotoxins (28), soybean trypsin inhibitor (0.4 mg/ml) was injected into each loop at the time of challenge. Following surgery, each animal was injected intramuscularly with butorphanol tartrate (0.2 mg/kg of body weight) to reduce discomfort. The duration of in vivo exposure to each enterotoxin was 2 h in all experiments, except for the data summarized in Fig. 1, in which intestinal exposure to CT was for 4 h. The toxin doses were determined by their effects on fluid accumulation and are indicated in the figure legends and Results. Animals were euthanized by an intravenous injection of sodium pentobarbitol (26%), and the volume of intestinal fluid in each loop was measured.

**Tissue preparation.** The intestinal fluid present in each loop was collected, along with a 10-ml saline lavage of empty loops. One-milliliter samples of the uncentrifuged intestinal fluid were mixed with 14.5 μl of 7 N perchloric acid and stored in amber microcentrifuge tubes at −70°C until the 5-HT content could be measured. The remaining intestinal fluid was clarified by centrifugation (1,500 × *g*) and stored at −70°C until cAMP, cGMP, and $PGE_2$ assays could be performed. The mucosa of each intestinal loop was scraped into 5 ml of cold phosphate-buffered saline with glass microscope slides and mixed immediately with an equal volume of cold 15% trichloroacetic acid in 0.2 N HCl to precipitate mucosal protein and to release intracellular contents. Samples were stored at 4°C until the mediators could be assayed, at which time the precipitated protein was dissolved in approximately 20 ml of 0.5 M KOH.

**Assays for intestinal mediators.** For measurement of 5-HT, perchloric acid extracts of intestinal fluid (200 μl) were injected into a high-performance liquid chromatograph (Waters; Milford, Mass.) fitted with a 5-μm-diameter $C_{18}$ column (Vydac, Hesperia, Calif.). The column was pumped at a rate of 1 ml/min with 26% pump A containing methanol. Pump B (74%) contained a solution (pH 2.75) of 0.05 M $Na_2HPO_4$, 0.03 M citric acid, 0.1 mM EDTA, and 0.042% sodium octyl sulfate, as described previously (5). On this column 5-HT eluted at 7.5 min, while other tryptophan metabolites, 5-hydroxytryptophan, 5-hydroxyindole acetic acid, tryptophan, and melatonin, typically eluted at 5.1, 5.5, 10.6, and 14.3 min, respectively. Standard amounts of 5-HT down to 20 pg were detected with a Waters 470 fluorescence detector programmed for an excitation wavelength of 285 nm and an emission wavelength of 340 nm. The $PGE_2$ and cGMP contents of the intestinal fluids were measured by radioimmunoassay, using kits purchased from Perseptive Diagnostics Inc. (Cambridge, Mass.). Samples were extracted as recommended by the manufacturer. The level of cAMP in mucosal tissue extracts and in intestinal fluid was assayed by a radiometric assay with protein kinase, as described previously (23). Mucosal tissue protein was estimated by the method of Bradford (3).

**Statistics.** Data were analyzed by an analysis of variance and subsequently by the Tukey test for multiple group comparisons or a *t* test within the analysis of variance. Unless specified otherwise, all *P* values were derived with the more stringent Tukey test.

## RESULTS

**Effects of CT, STa, and STb on intestinal fluid accumulation.** The effects of CT, STa, and STb on fluid accumulation are presented in Fig. 1A, 2A, and 3A, respectively. All doses of CT elicited fluid accumulation in 4 h that was significantly more than that in the control ($P < 0.05$). In addition, the volume of intestinal fluid accumulating in response to each enterotoxin was dose dependent. The selected doses of STa and STb were sufficiently high to achieve maximal stimulatory responses in a 2-h period (Fig. 2A and 3A); however, fluid accumulation responses with CT doses up to 64 μg/ml were large but somewhat less than maximal in 4 h (Fig. 1A). The intestinal fluid from the various toxin-treated loops was clear and devoid of blood on gross examination.

**Enterotoxin effects on the levels of intestinal mediators.** The $PGE_2$ levels in intestinal fluid from loops challenged with CT, STa, and STb are shown in Fig. 1B, 2B, and 3B, respectively. CT stimulation of $PGE_2$ synthesis and release into the intestinal fluid in cholera patients and in animal models has been described previously (2, 4, 20–22, 25, 26). Therefore, it was not surprising to detect significant release of this eicosanoid into the loop fluid in response to CT doses greater than 1 μg/ml ($P < 0.05$) (Fig. 1B). Similarly, we observed that $PGE_2$ release increased significantly in a dose-dependent manner from loops injected with an STb dose above 10 ng/ml ($P < 0.05$). In this study, CT released approximately two times more $PGE_2$ than did STb, but the toxin exposure times were 4 and 2 h, respectively. In contrast, we observed a minimal increase in $PGE_2$ release from STa-injected loops. $PGE_2$ responses to doses of STa above 10 ng/ml were considered significant by a *t* test within the analysis of variance ($P < 0.05$) but not by the Tukey test ($P > 0.05$).

The cAMP content of pig loops challenged with CT, STa, or STb is shown in Fig. 1C, 2C, and 3C, respectively. Without exception, no significant increase in cAMP above control levels ($P > 0.05$) was detected in extracts of the intestinal mucosal tissue exposed to the three bacterial enterotoxins. The cAMP content of the mucosa from intestinal loops exposed to each dose of CT appeared higher than that of controls, but the values were not statistically different from the control values ($P > 0.05$) and the responses were not related to CT dose (Fig. 1C).

The apparent refractory nature of pig intestinal mucosa to CT-mediated accumulation of cAMP was reported earlier (11, 14). Since the capacity of CT to stimulate adenylate cyclase in virtually all other cells was well known (18), we determined whether CT-induced cAMP might have escaped detection in pig intestinal mucosa by being extruded into the intestinal fluid (Fig. 4). The data indicated that all CT doses evoked significant fluid accumulation ($P < 0.05$) (Fig. 4A), and the release of cAMP from the intestinal mucosa into the luminal fluid was significantly increased in a dose-related manner ($P < 0.05$) (Fig. 4B).

Considering that we measured an increase in CT-induced cAMP in the intestinal fluid of pigs rather than in the mucosal tissue, it became important to assay the cAMP content of fluids from pig intestinal loops challenged with STa and STb. Figure 5A shows the fluid accumulation values resulting after a 2-h challenge of pig intestinal loops with two doses of STa (1.6 and 0.4 μg/ml) and STb (5.3 and 1.33 μg/ml). These doses of STa and STb were selected after numerous dose-response experiments, and the highest dose of either toxin was used to ensure that maximal secretory responses would be observed with each toxin administered separately. These experiments were performed with STa and STb administered alone and in combination, and fluid accumulation at all doses was significantly more than that in the control ($P < 0.05$). After the residual fluid volume of the control was subtracted, the effect of maximal response doses of STa and STb administered together yielded additive fluid volumes. The data in Fig. 5B and C indicate that no significant increases in cAMP could be detected in mucosal extracts or in luminal fluids from loops challenged with STa or STb ($P > 0.05$).

STa increased the release of cGMP into the luminal fluid (Fig. 6A), although no increase in mucosal cGMP was observed (Fig. 6B). During the 2-h observation period, the STa-induced increase in cGMP was minimal but significant ($P < 0.05$) when evaluated by the *t* test within the analysis of vari-

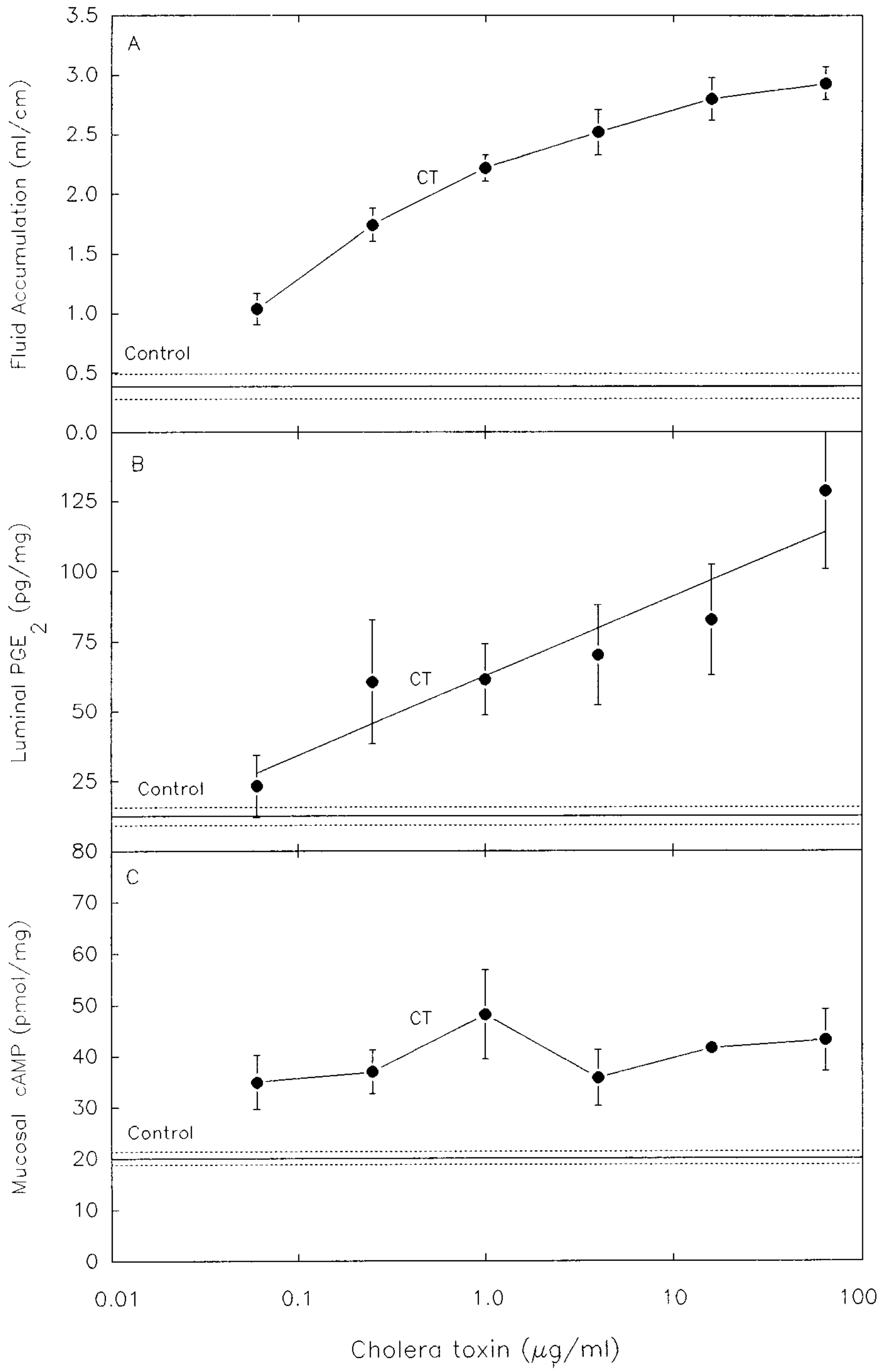


FIG. 1. Effect of CT dose on intestinal responses in the pig intestinal loop model. (A) Mean volumes of fluid accumulating in intestinal loops from six pigs after 4 h; (B) amount of $PGE_2$ released into the luminal fluid; (C) cAMP levels in mucosal tissue. The vertical bars and dotted lines reflect 1 standard error above or below the mean.

ance. In contrast, no effect of STb on cGMP was apparent ($P > 0.05$) (Fig. 6). When intestinal loops were challenged with a mixture of STa and STb, the concentration of cGMP in the intestinal fluid was comparable to that of the control ($P > 0.05$) (Fig. 6A). No increases in cGMP were detectable in mucosal tissue extracts from loops injected with STa and/or STb ($P > 0.05$) (Fig. 6B).

The intestinal $PGE_2$ and 5-HT responses of pigs challenged with STa and/or STb are illustrated in Fig. 7A and B, respectively. The $PGE_2$ data (Fig. 7A) confirm earlier data, shown in Fig. 3B, that STb is similar to CT (Fig. 1B) in exerting significant stimulatory effects on $PGE_2$ synthesis (high dose: $P < 0.05$ by Tukey test) (low dose: $P < 0.05$ by *t* test). In contrast, STa has a minimal effect on $PGE_2$ release from pig mucosa ($P < 0.05$, *t* test) (Fig. 2B). In a manner similar to that reported for CT (7, 19), STb caused significant release of 5-HT at both

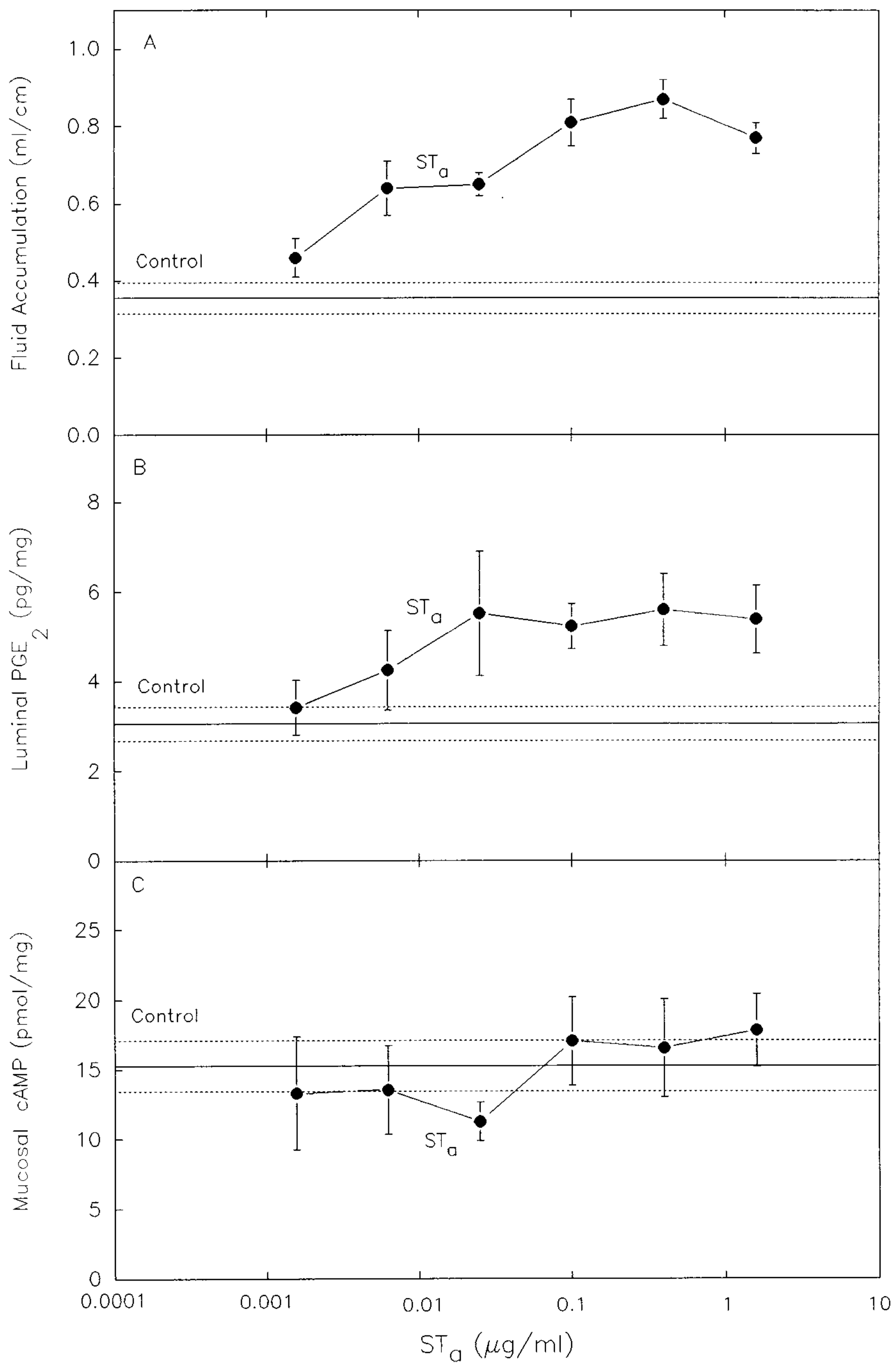


FIG. 2. Effect of *E. coli* STa dose on intestinal responses in the pig intestinal loop model. (A) Mean volumes of fluid accumulating in intestinal loops from six pigs after 2 h; (B) amount of $PGE_2$ released into the luminal fluid; (C) cAMP levels in mucosal tissue. The vertical bars and dotted lines reflect 1 standard error above or below the mean.

doses ($P < 0.05$), whether alone or in combination with STa, whereas STa exerted no effect on 5-HT levels ($P > 0.05$) (Fig. 7B). The effect of these toxins on the release of 5-HT from EC cells into the intestinal fluid appears to correlate closely with their effects on $PGE_2$ levels (Fig. 7A).

The data in Table 1 show the effect of combining CT and STb on fluid accumulation and release of 5-HT into the pig intestinal lumen within a 2-h observation period. Maximal doses of STb (5.3 μg/ml) combined with high doses of CT (64 μg/ml) yielded additive effects on both fluid accumulation and 5-HT release.

## DISCUSSION

In this report, we used the pig intestinal loop model to compare the secretory effects of CT, STa, and STb. By measuring intestinal fluid accumulation, as well as the levels of cAMP, cGMP, $PGE_2$, and 5-HT in the intestinal samples, we

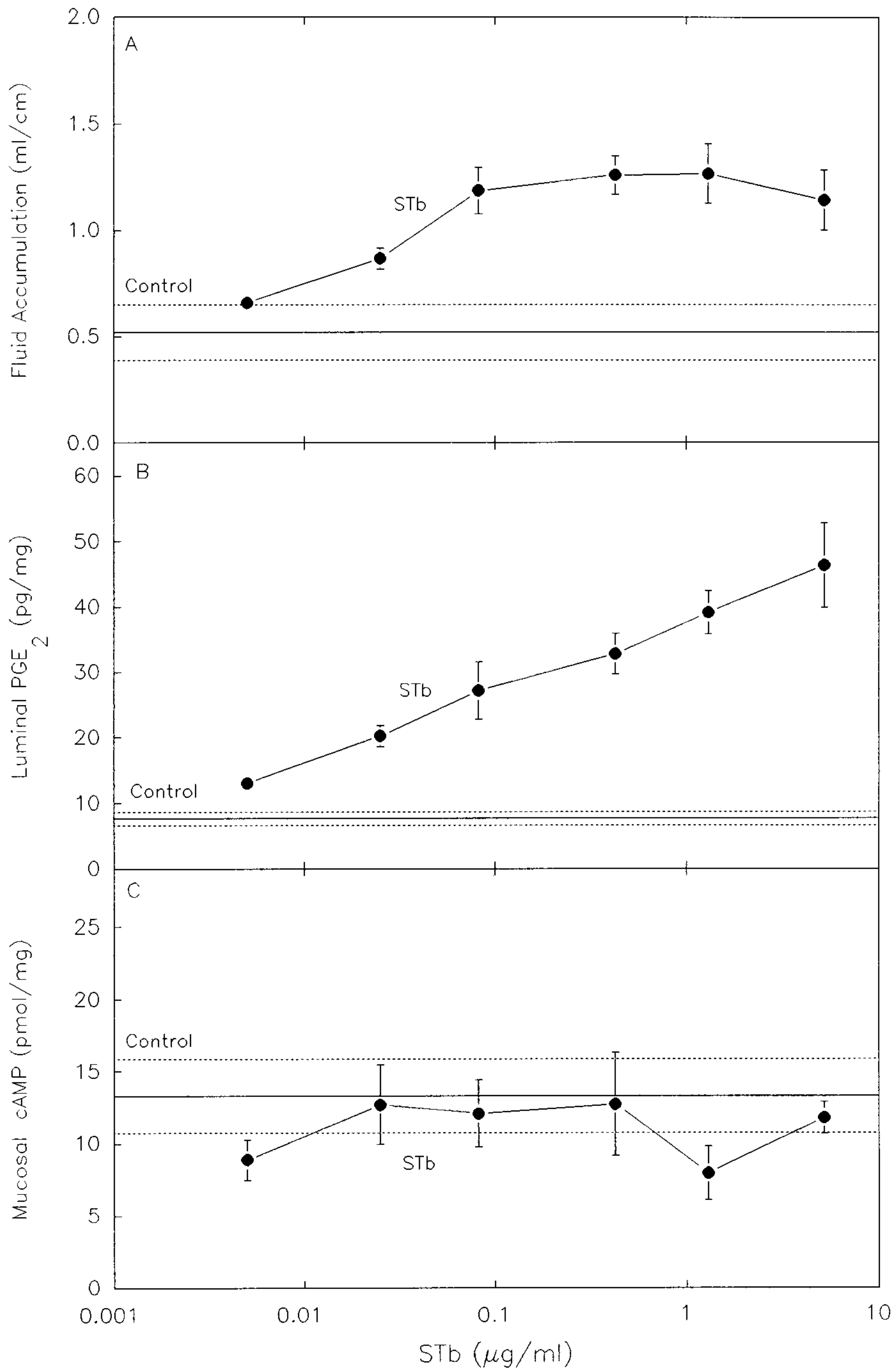


FIG. 3. Effect of STb dose on intestinal responses in the pig intestinal loop model. (A) Mean volumes of fluid accumulating in intestinal loops from six pigs after 2 h; (B) amount of $PGE_2$ released into the luminal fluid; (C) cAMP levels in mucosal tissue. The vertical bars and dotted lines reflect 1 standard error above or below the mean.

made several observations about the mechanisms of action of the toxins. First, the potencies of the three enterotoxins could not be compared with accuracy in these studies, because the optimum times for inducing secretion by each toxin were not the same. For, example, STa and STb are known to elicit a rapid secretory response of brief duration, while CT evokes a delayed and prolonged response. All of our data (except for those in Fig. 1) were derived after a 2-h exposure of pig intestinal loops to each toxin. The CT dose-response experiment (Fig. 1) involved a 4-h observation period. Linear regression analysis (Fig. 2 and 3) indicated the doses of STb and STa that would evoke fluid accumulation of approximately 1 ml/cm in 2 h to be 35 pmol (38 ng/ml) and 60 pmol (24 ng/ml), respectively. The low molar doses of STb and STa necessary for this enterotoxic response could be attributed to the rapid action of these toxins and to their low molecular weights. In comparison, high concentrations of CT (e.g., 3,810 pmol [64 μg/ml]) were necessary to evoke a similar fluid accumulation response (0.8

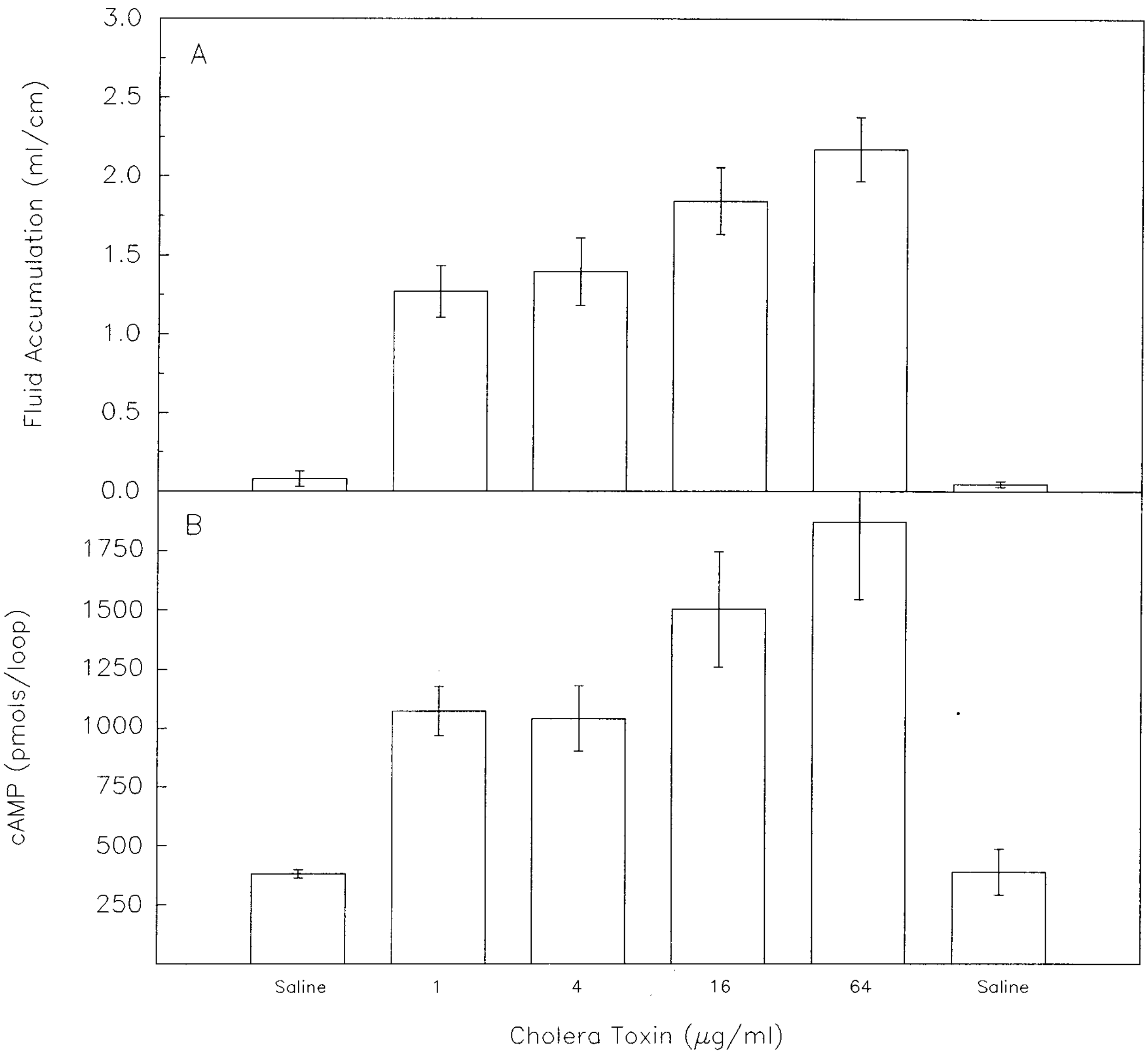


FIG. 4. Amounts of CT-induced fluid (A) and cAMP (B) secreted into the lumen of pig intestinal loops after 2 h. The values indicate the mean responses from six pigs, and the vertical bars reflect 1 standard error above or below the mean.

ml/cm) in 2 h (Table 1). While STa and STb secretory responses decreased after 2 h (data not shown), the CT response increased markedly, and by 4 h the dose of CT that elicited fluid accumulation of 1 ml/cm was 3.1 pmol (52 ng/ml) (Fig. 1). Thus, CT could be considered the most potent of the three enterotoxins, but additional time is required to observe its actual effect on intestinal secretion.

Second, we observed that coinjection of maximal-response doses of both STa and STb into pig intestinal loops yielded additive fluid accumulation (Fig. 5A). Since STa and STb induced maximal secretory responses individually and additive responses when combined, one interpretation of this observation is that these two enterotoxins have different mechanisms of action. Combining maximal doses of toxins having similar mechanisms of stimulating intestinal secretion would have been expected to yield no additional fluid accumulation. We also noted that STa-induced cGMP levels (Fig. 6A) were diminished when STa was injected with STb; however, the importance of this observation is unknown at this time. In a similar experiment, we tested combined doses of CT and STb. Data in Table 1 indicated that near-maximal doses of CT and STb evoked additive fluid accumulation and 5-HT release responses. We concluded from both of these experiments that CT, STa, and STb possess different mechanisms of action, which was not altogether unexpected. Nevertheless, analysis of the various intestinal mediators and second messengers revealed both similarities and differences as described below.

The synthesis of mediators in intestinal loops exposed to CT, STa, and STb varied from one toxin to the other. CT and STb elicited dose-related increases in the synthesis of $PGE_2$ (Fig. 1B and 3B), a paracrine hormone capable of stimulating electrolyte transport. STa had little or no effect on $PGE_2$ synthesis (Fig. 2B and 7A). Because of the complexity and number of cell types in the intestine, the source of the CT- and STb-induced prostaglandins and their site of action are not known.

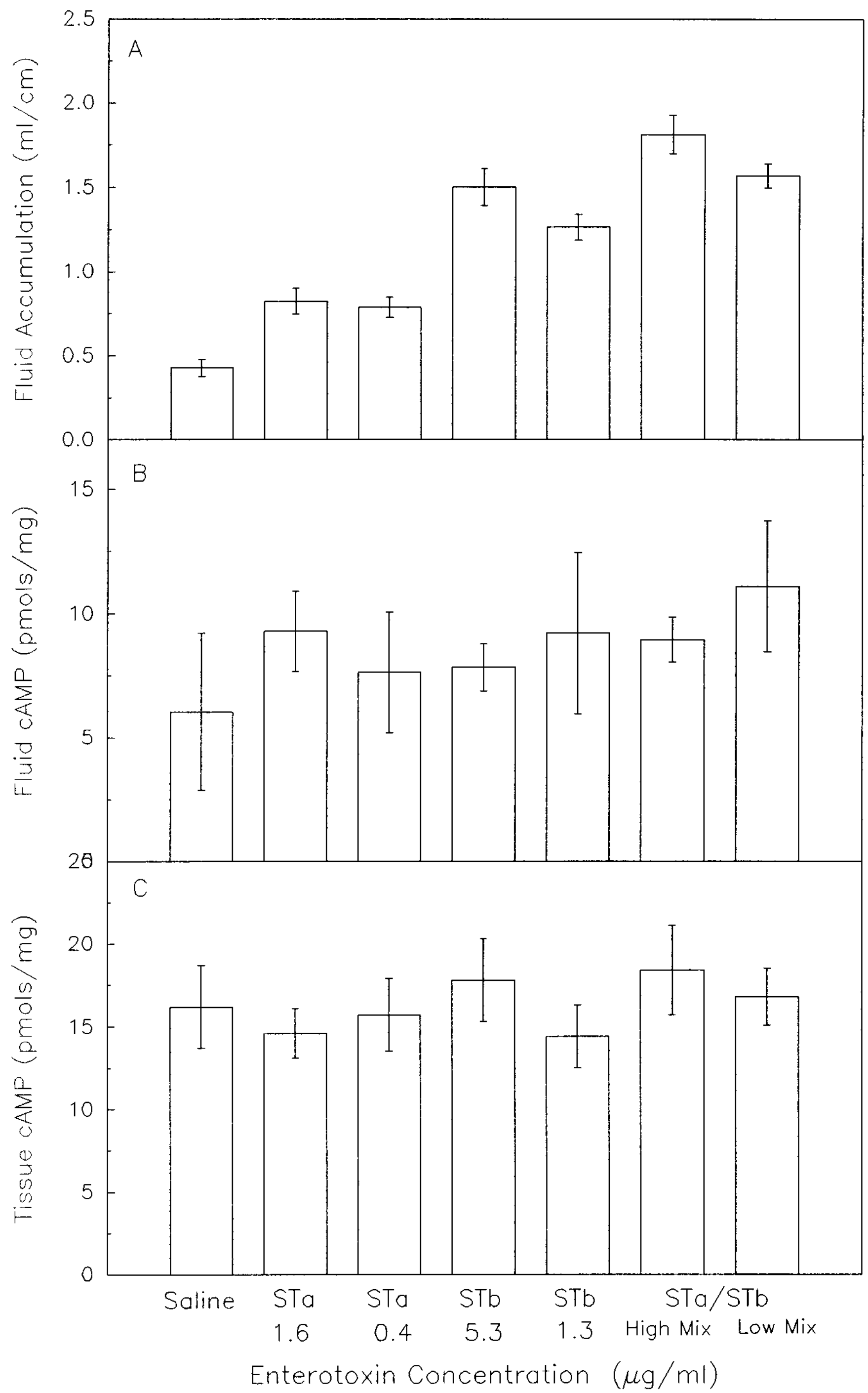


FIG. 5. Effects of STa and STb on fluid accumulation (A) and cAMP levels in pig intestinal loop fluid (B) and tissue (C) in 2 h. The highest dose of STa was 1.6 μg/ml (5 ml), and the lowest dose tested was 0.4 μg/ml (5 ml). In contrast, STb was tested at 5.3 μg/ml (5 ml) and 1.3 μg/ml (5 ml). These doses were selected from dose-response experiments to ensure that maximal secretory effects of each toxin would be observed at the highest dose. cAMP results are expressed per milligram of tissue protein. The values indicate the mean responses from six pigs, and the vertical bars reflect 1 standard error above or below the mean.

Nevertheless, receptors for these toxins are located on the surface of the epithelium, and the toxins are not known to reach the lamina propria. Epithelial cells and EC cells, exposed to the lumen, could release mediators that interact with other cell types in the tissue below the epithelium. In fact, substantial evidence that the enteric nervous system, a network of neurons that lie under the epithelium, has an important role in cholera pathogenesis has accumulated (7, 8, 19). In this process, enterotoxic signals are transferred from the EC cells to the crypt epithelial cells and to circular smooth muscle via subepithelial neurons. The process can be initiated by the release of 5-HT from the EC cells by stimulation of cAMP synthesis, as in the case of CT. Although we could not demonstrate any effect of STb on intestinal cAMP levels, STb induced the release of both

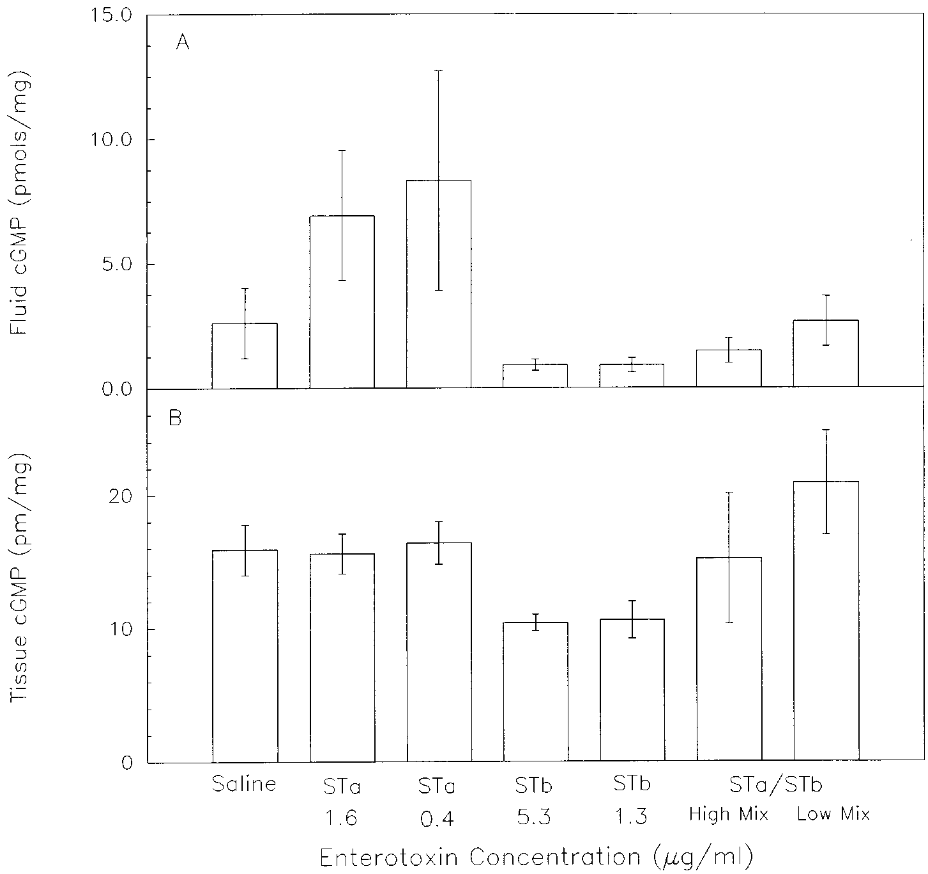


FIG. 6. cGMP responses of pig intestinal loop fluid (A) and tissue (B) after 2-h exposure to STa and/or STb. The doses of STa and STb are defined in the legend to Fig. 5. Results are expressed as picomoles per milligram of mucosal protein. The values indicate the mean responses from six pigs, and the vertical bars reflect 1 standard error above or below the mean.

$PGE_2$ and 5-HT in a manner similar to that of CT, suggesting a mechanism of action involving the enteric nervous system.

We were able to explain past observations (11, 14) that CT did not appear to elevate cAMP levels in pig intestinal mucosa (Fig. 1C). Our data indicated a minimal, but statistically insignificant, increase in cAMP concentration in mucosal tissues at all CT doses, although no dose relationship was apparent (Fig. 1C). In fact, cAMP was efficiently extruded into the intestinal lumen (Fig. 4), leaving some residual cAMP in the CT-treated tissues. These data suggested that the pig intestine might be more efficient in modulating intracellular cAMP levels by extrusion compared with other animals, such as the rabbit, in which cAMP extrusion has been documented (20, 22). For toxins that have no apparent effect on cyclic nucleotide levels, such as STb, other toxin-generated stimuli might serve to invoke EC cell release of 5-HT (Fig. 7B). We also observed that STb stimulated the synthesis and release of $PGE_2$ from the intestinal mucosa. $PGE_2$ is capable of stimulating cAMP formation by adenylate cyclase, which in turn could result in the release of 5-HT from EC cells into the intestinal lumen. Although we could not demonstrate that STb caused cAMP synthesis, small immeasurable increases in EC cell cAMP, caused by STb-induced $PGE_2$ synthesis, could have been responsible for the observed release of 5-HT (Fig. 7B). The stimulatory effect of STb on the release of mucosal $PGE_2$ and 5-HT was similar to that reported for CT in the rat, cat, and rabbit models (2, 19, 20, 22).

In conclusion, the molecular responses of porcine intestine to each bacterial enterotoxin were unique, despite a basic stimulatory effect on intestinal secretion. A summary of the effects of CT, STa, and STb on pig intestine is presented in Table 2. The data support a potential role of cAMP and cGMP in the mechanism of action of CT and STa, respectively, as has been discussed for many years. These second messengers might act in concert with other molecules (e.g., 5-HT release from intestinal EC cells) to activate the enteric nervous system. Despite the complexity of these cellular events and their impact on the physiologic response of the intestinal mucosa, it is intriguing to consider the potential similarity of CT and STb action (Table 2). While STb's mechanism of action is poorly understood, it is clear that STb does not increase either cGMP or cAMP (15) (Figs. 2, 3, and 6). We noted in this study that STb caused a dose-dependent increase in $PGE_2$ release, which was similar to that reported for CT (2, 4, 21, 22, 25, 26). It is not clear how two dissimilar enterotoxins could stimulate $PGE_2$ synthesis. Although the amino acid sequences and molecular sizes of CT and STb are quite different, it is possible that $PGE_2$ has an integral role in CT- and STb-induced secretion. STa's effects on the synthesis of these mediators seemed quite different from that of CT and STb, because it was limited to effects on cGMP (Table 2).

STb's stimulatory effect on 5-HT release from intestinal EC cells in the pig intestine (Fig. 7B) was strikingly similar to that of CT (Table 1) (2, 7, 19, 20, 22). In the case of CT,

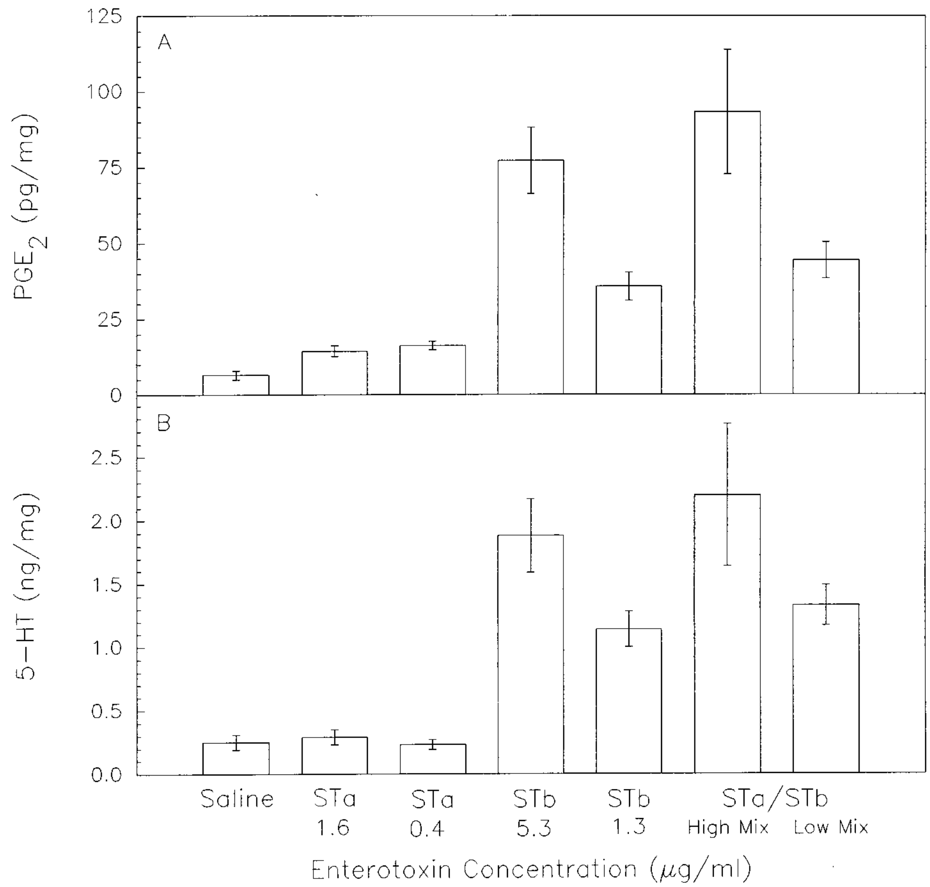


FIG. 7. $PGE_2$ and 5-HT responses measured in luminal fluid from pig intestinal loops challenged for 2 h with STa and/or STb. The doses of STa and STb are defined in the legend to Fig. 5. Results are expressed per milligram of mucosal tissue protein from which the substances were secreted. The values indicate the mean responses from six pigs, and the vertical bars reflect 1 standard error above or below the mean.

strong evidence supports the concept that elevation of cAMP in intestinal EC cells causes them to release 5-HT, which in turn, initiates nerve impulse transmission through the enteric nervous system (8). Because STb evokes a potent $PGE_2$ response, it is possible that this eicosanoid either causes the concomitant release of 5-HT from the intestinal EC cells or is involved in the signal transduction process. It is unknown at this time whether $PGE_2$ acts alone or gives rise to immeasurable increases in cAMP in intestinal cells, which could trigger the release of 5-HT. Regardless, the available data suggest that STb and CT exert a common effect on the enteric nervous system. Along these lines, STa already has been shown to stimulate the enteric nervous system (9), presumably via its effects on cGMP levels. While each enterotoxin elicits the synthesis or release of unique mediators, intestinal secretion may culminate from the impact of these substances on neural control of intestinal physiology, rather than on their direct impact on ion channel activity. Through continued study of bacterial enterotoxins and their mechanisms, eventually it might be possible to identify specific points in intestinal metabolism or signal transduction where the sequence of molecular events could be interrupted or reversed to control intestinal secretion of water and electrolytes. Consequently, such strategies could lead to improvements in clinical control of dehydration and a reduction in diarrheal disease patient mortality regardless of microbial etiology.

TABLE 1. Additive effects of CT and STb on fluid accumulation and luminal release of 5-HT in pig intestinal loops

| Loop content (μg/ml) | Fluid accumulation (ml/cm ± SD) | Luminal 5-HT (ng ± SD) |
|---|---|---|
| Saline control | 0.29 ± 0.05 | 161 ± 45 |
| CT (16) | 0.61 ± 0.08 | 232 ± 48 |
| CT (64) | 0.80 ± 0.04 | 265 ± 70 |
| STb (1.3) | 0.90 ± 0.12 | 359 ± 80 |
| STb (5.3) | 0.84 ± 0.11 | 398 ± 92 |
| CT (16) + STb (1.3) | 1.14 ± 0.10 | 390 ± 105 |
| CT (64) + STb (5.3) | 1.19 ± 0.10 | 575 ± 185 |

TABLE 2. Summary of enterotoxin effects on pig intestine[a]

| Effect | CT | STb | STa |
|---|---|---|---|
| Fluid accumulation | + | + | + |
| Release of 5-HT | + | + | − |
| Increased $PGE_2$ | + | + | ± |
| Increased cAMP | + | − | − |
| Increased cGMP | − | − | + |

[a] +, effect observed; −, effect not observed; ±, low levels observed (Fig. 2B).

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