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Some Properties of Purified *Escherichia coli* Heat-Stable Enterotoxin II

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We examined the biological properties of purified *Escherichia coli* heat-stable enterotoxin II (STII) using mouse intestinal loop assays and compared these properties with those of heat-stable enterotoxin I (STI) and cholera toxin (CT). The action of STII over time differed from those of STI and CT. STII did not alter cyclic GMP or cyclic AMP levels in intestinal mucosal cells. Our results supported the idea that the mechanism of action of STII in inducing fluid secretion is different from the mechanisms of action of STI and CT. This hypothesis was further supported by the fact that an anti-STII neutralizing serum did not neutralize the activities of STI and CT. Subsequently, we examined the involvement of prostaglandins in the action of STII. The level of prostaglandin E_2 in the fluid accumulated as a result of the action of STII increased, and the prostaglandin synthesis inhibitors aspirin and indomethacin significantly reduced the response to STII. These results implicate prostaglandin E_2 in the mechanism of action of STII.

Enterotoxigenic *Escherichia coli* causes diarrhea in newborn animals and in children in less-developed countries and is also the agent most frequently responsible for traveler's diarrhea in these countries (20, 28). Two classes of enterotoxin, heat-labile enterotoxin and heat-stable enterotoxin, have been implicated in the pathogenesis (1, 10, 38). The former resembles cholera toxin (CT) not only functionally but also structurally and immunologically (4, 8, 15, 21, 36, 37).

The heat-stable enterotoxins have been classified into two groups on the basis of their physiological and biological properties (2): STI (also referred to as STa) and STII (also referred to as STb). STI is active in a suckling mouse assay and has been proved to be an 18- or 19-amino-acid acidic peptide that contains three disulfide bonds (11, 30, 31, 33, 34). The physicochemical and biological properties of STI have been well studied (22–24). It has been proposed that STI binds to specific receptors located on the brush border of small intestinal enterocytes (29). The binding induces an increase in cyclic GMP levels through the activation of a particular guanylate cyclase activity. The increase in cyclic GMP levels leads to net chloride and fluid secretion (12, 26).

On the other hand, STII has been reported to be active only in a piglet intestinal loop assay (2, 14). The purification of STII was not achieved until quite recently because of the lack of a convenient toxin assay. Recently, Whipp demonstrated that the host response specificity of STII could be attributed to the susceptibility of STII to protease and that STII also was active in a rat intestinal loop assay in the presence of a protease inhibitor (39, 40). More recently, we purified STII and demonstrated that it is composed of 48 amino acid residues that contain two disulfide bonds, between Cys-10 and Cys-48 and Cys-21 and Cys-48 (7). However, the biological properties of STII have remained unclear. In this study, we examined some biological properties of purified STII and compared these properties with those of STI and CT.

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MATERIALS AND METHODS

Toxin preparation. STII was purified from the culture supernatant of *E. coli* HB101(pCHL7) by successive column chromatographies as described previously (7). CT and STI were purchased from Sigma Chemical Co. (St. Louis, Mo.).

Mouse intestinal loop assay. Unless otherwise stated, the enterotoxin activities of samples were assayed in a mouse intestinal loop assay.

Mice weighing 30 to 35 g were used. The mice were anesthetized with sodium pentobarbital, and the intestines were exteriorized through a midline incision. The intestinal lumen was rinsed three times with saline containing 100 U of aprotinin per ml. After the rinses, a series of ligated intestinal segments (loops), about 4 cm long and separated by a 0.5- to 1-cm interloop, were created. The most proximal loop was placed about 4 cm distal to the ligament of Treitz. One or two loops were created per intestine. Each loop was injected with 0.2 ml of toxin solution. After an appropriate incubation period, the mice were killed and the loop(s) was removed from the carcass. The sample activity was expressed as weight of the loop (in grams)/length of the loop (in centimeters).

Suckling mouse assay. The suckling mouse assay was performed as described previously (22, 23). In brief, 0.1-ml samples were administered via a gastric tube to the stomach of 2- to 3-day-old suckling mice, with 0.001% Evans blue dye as a marker. The mice were killed 2.5 h later, and ratios of intestinal weight to body weight were determined. An intestinal/body weight ratio of 0.083 was considered positive response. The minimal amount of ST producing a positive response was designated 1 U, and the enterotoxin titer was expressed as the reciprocal of the highest dilution that produced 1 U of enterotoxin activity.

Antibody production. Three milligrams of purified STII was conjugated with 5 mg of keyhole limpet hemocyanin (Calbiochem Corp., La Jolla, Calif.). The conjugate was emulsified with complete Freund's adjuvant, and the emulsion was injected into footpads of guinea pigs. Injections were performed three times at 2-week intervals. Antiserum

was obtained 2 weeks after the final injection and inactivated by being heated at 56°C for 30 min.

Neutralization of enterotoxin activity. Anti-STII serum was diluted two times in succession with phosphate-buffered saline (PBS) containing purified STII (5.6 μg/ml), and each solution was incubated at 37°C for 1 h. As described in Results, STII can express its activity in the presence of a protein inhibitor in the mouse intestinal loop assay. Therefore, 0.18 ml of the incubated solution that contained 1 μg of STII was mixed with 0.02 ml of aprotinin solution (25,000 U/ml; Bayer, Leverkusen, Germany). A 0.2-ml quantity of the mixture was injected into a mouse intestinal loop to examine the neutralizing effect of the serum. Normal guinea pig serum inactivated by being heated at 56°C for 30 min was used as the control.

Neutralization of the activities of STI and CT by anti-STII serum was also examined. The procedures used for the neutralization of these toxins were almost the same as the procedure described above. STI activity and CT activity in the samples were determined by the suckling mouse assay and the mouse intestinal loop assay, respectively.

Accumulation of cyclic nucleotides. The effect of STII on the level of cyclic nucleotides in the mouse intestine was examined. STII was dissolved in aprotinin solution (2,500 U/ml), and the solution was injected into the intestinal loops of mice or into suckling mice as described above.

Mouse intestinal loops were removed after 3 h of incubation. The loops were immediately cut open along their lengths, and the mucosa was scraped by drawing a glass microscope slide over it. The mucosal scraping was immediately placed in 1.0 ml of 5% trichloroacetic acid (TCA), and the mixture was allowed to stand overnight at 4°C. After centrifugation (4,000 × *g* for 15 min), the pellet was dissolved in 1.0 N NaOH, and the solution was used for protein determination by the method of Lowry et al. (18). The supernatant was washed five times with water-saturated diethyl ether to remove TCA. After the excess diethyl ether was boiled off at 60°C, the aqueous phase was evaporated to dryness under a vacuum. The dried residue was resuspended in 50 mM Tris-HCl buffer (pH 7.5) containing 4 mM EDTA, and the suspension was assayed at appropriate dilutions for cyclic nucleotide content. Cyclic GMP and cyclic AMP contents were measured with commercially available radioimmunoassay kits (cyclic AMP and cyclic GMP assay systems; Amersham International plc, Amersham, United Kingdom).

Suckling mice were killed 3 h after the toxins were administered. The whole intestines were removed and homogenized in 1 ml of 5% TCA at 0°C. The homogenates were subjected to almost the same treatment as that described above.

Prostaglandin measurement. The amounts of prostaglandin E_2 (PGE_2) released into mouse intestinal lumens after exposure to STII (2 μg per loop) and CT (1 μg per loop) were determined. Mouse intestinal loops were incubated either for 8 h with CT or for 3 h with STII. The fluid accumulated in the loops was removed, and the volumes of the accumulated fluid were determined. The mean volumes of fluid accumulated per loop with STII and CT were 1.2 and 1.5 ml, respectively. Control loops for the STII experiments were inoculated with 0.2 ml of PBS containing aprotinin (2,500 U/ml) and incubated for 3 h. After incubation, the contents in the control loops were washed out with 1.2 ml of PBS. Control loops for the CT experiments were inoculated with 0.2 ml of PBS and incubated for 8 h. After incubation, the intestinal contents were washed out with 1.5 ml of PBS. The volume of PBS used for the washing was proportional to the volume of fluid accumulated in the loops with each toxin.

A 0.5-ml portion of this fluid was combined with 0.1 ml of 1 N HCl containing 50% TCA. The resulting precipitates were separated from the supernatant by centrifugation. The pellets were dissolved in 1.0 N NaOH, and the solution was assayed for protein content by the method of Lowry et al. (18). Three milliliters of water-saturated ether was added to the supernatant, and the mixture was agitated for 1 min. The organic phase was discarded, and 3 ml of an ethyl acetate–isopropanol–0.2 N HCl solution (3:3:1 [vol/vol/vol]) was added to the aqueous phase. The mixture was vortexed for 15 s twice, and 2 ml of ethyl acetate and 3 ml of water were added. After mixing was done, the phases were separated by centrifugation, and the obtained organic phase was lyophilized in a vacuum centrifuge (Tomy Seiko Co., Tokyo, Japan). The PGE_2 concentration in each sample was determined with a commercially available radioimmunoassay kit (bicyclic PGE_2 assay system; Amersham). The amount of PGE_2 in intestinal fluid was expressed as picomoles of PGE_2 per milligram of protein.

Effects of pharmacologic agents. The effects of indomethacin and aspirin on the activities of STII were examined with the mouse intestinal loop assay. Mice were injected intraperitoneally with aspirin (50 mg/kg; Sigma) or with indomethacin (50 and 25 mg/kg; Sigma) 3 h before the injection of STII. Control mice received an identical volume of 10 mM Tris-HCl buffer containing 0.9% NaCl. Intestinal loops from these mice were prepared as described above. Each loop was injected with 0.2 ml of a solution containing STII (2.5 μg/ml) and aprotinin (2,500 U/ml). All experimental and control mice were killed 3 h after the toxin challenge, and the amounts of fluid accumulated in the loops were determined.

Intestinal histopathology. After incubation of 1 μg of purified STII for 3 h in a mouse intestinal loop, a portion of the loop was removed and immediately fixed in 10% buffered formalin. The loop portion was sectioned and stained with hematoxylin and eosin.

RESULTS

Effects of a protease inhibitor and washing on the response to STII. It is known that STII cannot induce a positive response in the absence of a protease inhibitor in the rat intestinal loop assay because STII administered to rat intestinal loops is degraded by intestinal proteases (39, 40). The response to purified STII in the mouse intestinal loop assay was also negative in the absence of a protease inhibitor (data not shown). However, we found that the response became positive in the presence of a protease inhibitor (Fig. 1). We used aprotinin as the protease inhibitor. In further experiments, 500 U of aprotinin was injected per loop for the determination of the activity of STII in the mouse intestinal loop assay.

The effect of washing of the mouse intestinal lumens on the response to STII was examined. The intestinal lumens were washed before preparation of the intestinal loops by the procedure described in Materials and Methods. The amounts of STII injected into loops made from washed intestines and into loops made from unwashed intestines are indicated in Fig. 1. The amounts of fluid accumulated in the loops were determined 3 h after the injections. The loops made from washed intestines were more sensitive to STII than the loops made from unwashed intestines (Fig. 1); a response of 0.210 g/cm was observed after the injection of 0.5 μg of purified STII into loops created from washed intestines, but 10 times

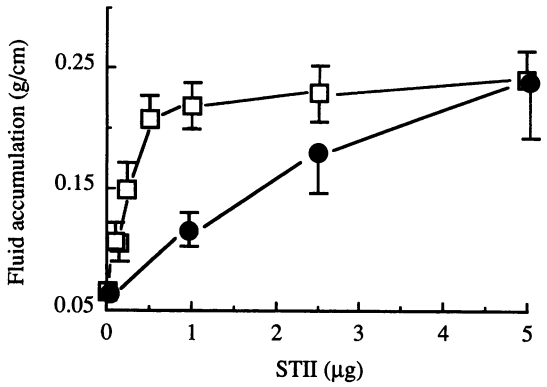

FIG. 1. Effect of washing of intestinal lumens on the response to STII. Mice were anesthetized, and the intestines were exteriorized. Intestinal lumens of half of the mice (□) were rinsed with saline containing 100 U of aprotinin per ml and those of the remaining half of the mice (●) were not rinsed. These intestines were ligated to create loops, and each of the created loops was injected with the indicated amount of STII in the presence of aprotinin as described in the text. Three hours after the injections, the mice were killed and the activities of the samples were measured and expressed as weight of the loop (in grams)/length of the loop (in centimeters). Values represent means ± standard errors for five determinations.

as much STII (5 μg) was required to cause the accumulation of the same volume of fluid in loops created from unwashed intestines.

Response over time. The action of STII over time was examined with mouse intestinal loop assays (Fig. 2A). Each loop was injected with 0.2 ml of a solution containing 500 ng of purified STII. The fluid accumulated in each loop was determined at the times indicated in Fig. 2A. Fluid accumulation was observed as early as 30 min after the injection of STII, and the volume of fluid accumulated was maximal 3 h after the injection. Upon longer incubation, the volume of fluid accumulated gradually decreased, and the reaction was negative 16 h after the injection of STII.

The actions of STI and CT over time were also examined (Fig. 2B and C). Aprotinin was not injected into loops, because these two toxins did not require the protease inhibitor to evoke a secretory response. The amounts of STI and CT injected into each loop were 100 and 500 ng, respectively. In the loops injected with STI, fluid accumulation was observed starting 30 min after the injection, and the volume was maximal 2 h after the injection. The early action of STI is similar to that of STII, but almost all the fluid accumulated as a result of the action of STI disappeared from the loops 8 h later. The action of CT over time was quite different from that of STII. Measurement of fluid accumulation began 3 h after the injection of CT, and the amount of fluid accumulated reached a maximum 8 h after the injection. During the 16 h of observations, there never was a significant decrease in fluid accumulation.

Comparison of enterotoxin activities. The dose responses to three kinds of enterotoxin, STI, STII, and CT, in the mouse intestinal loop assay were examined. Mice were killed at the times when maximal fluid accumulation was observed, i.e., 2 h after STI injections, 3 h after STII injections, and 8 h after CT injections (Fig. 2). The loops were removed from the carcasses, and the amounts of fluid in the loops were determined as described in Materials and Methods (Fig. 3). The amounts of STI, STII, and CT that resulted in a fluid accumulation of 0.13 g/cm (putative

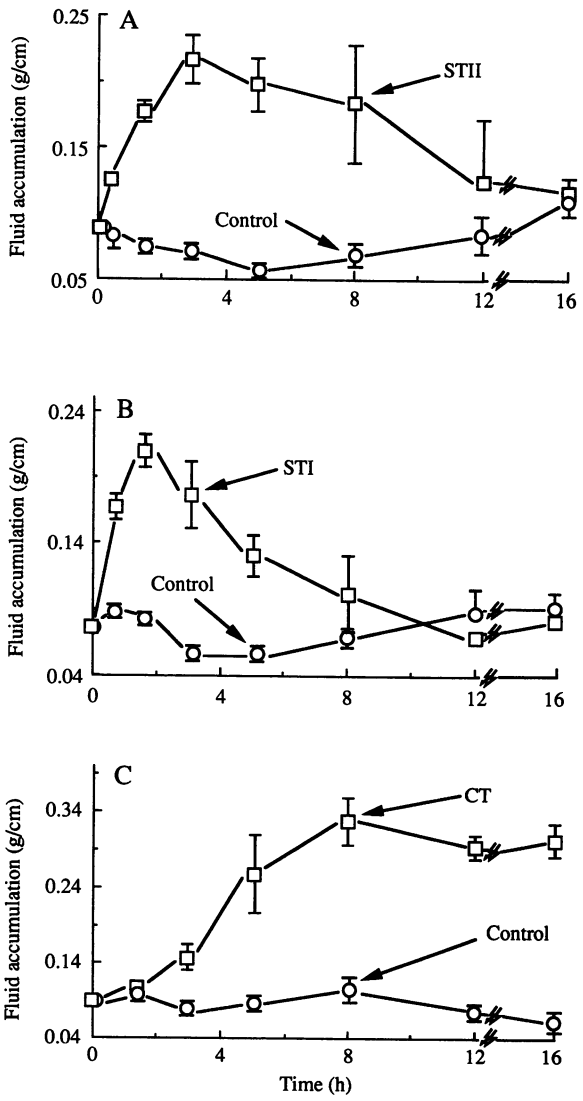

FIG. 2. Fluid accumulation caused by STII, STI, and CT over time. The actions over time of STII (A), STI (B), and CT (C) in mouse intestinal loops were examined. The amounts of purified STII, STI, and CT injected into the loops were 500, 50, and 500 ng, respectively. Control loops were injected with the same buffer that was used to dissolve each toxin. The toxic activities of the samples were determined at the indicated times as weight of the loop (in grams)/length of the loop (in centimeters). Values represent means ± standard errors for five determinations.

positive response ratio) were 6, 200, and 200 ng, respectively. These results indicate that the toxin possessing the strongest activity per unit mass among these three toxins is STI and that the toxicity of STII is almost the same as that of CT.

Neutralization of enterotoxin activities. A guinea pig antiserum developed against the conjugate of purified STII and keyhole limpet hemocyanin was obtained. The antiserum was diluted two times in succession with PBS containing STII, and each solution was incubated. After incubation, 0.2 ml of the mixture containing 1 μg of STII was injected into mouse intestinal loops to examine the ability of the antiserum to neutralize STII activity as described in Materials and Methods. Normal guinea pig serum was used as a control.

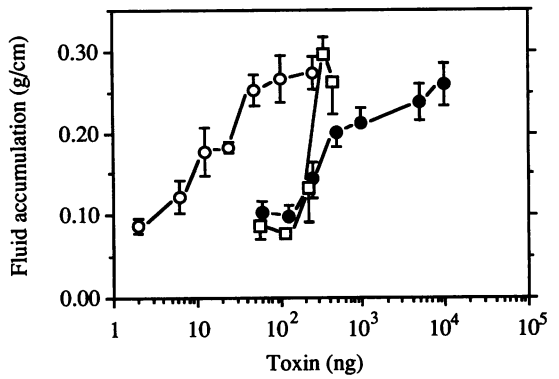

FIG. 3. Dose responses to purified STI, STII, and CT in the mouse intestinal loop assay. The indicated amounts of purified toxins were injected into intestinal loops and incubated for 2 h for STI (○), for 3 h for STII (●), and for 8 h for CT (□). Values represent means ± standard errors for five determinations.

TABLE 1. Effect of STII on cyclic nucleotide levels in mouse intestines

Mice	Treatment (amt of toxin administered)	Level (pmol/mg of protein) of:	
		Cyclic AMP	Cyclic GMP
Suckling	Control	5.68 ± 0.19	1.60 ± 0.28
	STII (5 μg)	5.37 ± 0.19	1.49 ± 0.15
	STII (50 μg)	6.03 ± 0.28	1.98 ± 0.30
	STI (5 ng)	5.38 ± 1.14	2.99 ± 0.19[a]
	STI (50 ng)	5.75 ± 0.96	7.57 ± 0.86[b]
Adult	Control	5.03 ± 0.83	0.90 ± 0.18
	STII (1 μg)	5.49 ± 0.39	0.84 ± 0.30
	STII (5 μg)	6.66 ± 1.58	0.92 ± 0.27
	CT (0.35 μg)	8.51 ± 1.43[b]	0.89 ± 0.11
	CT (1.75 μg)	12.36 ± 1.26[c]	0.87 ± 0.21

[a] $P < 0.05$ (versus control).
[b] $P < 0.03$ (versus control).
[c] $P < 0.01$ (versus control).

This anti-STII serum neutralized STII activity (Fig. 4); the antiserum diluted to 1/8 strength had enough activity to neutralize the activity of 1 μg of STII, but the antiserum diluted to 1/16 strength did not.

The ability of the anti-STII serum to neutralize STI and CT was examined by the same method. Anti-STII serum was diluted with PBS containing STI (150 ng/ml) or CT (4 μg/ml). After incubation, the mixture was administered either to suckling mice to detect STI activity or to mouse intestinal loops to detect CT activity. The amounts of STI and CT administered to suckling mice and to mouse intestinal loops were 7.5 and 400 ng, respectively. Neither of these activities was neutralized (data not shown).

Effect of STII on cyclic nucleotide levels in mouse intestines. Table 1 shows the effect of STII on cyclic AMP and cyclic GMP accumulation in mouse intestines. STI and CT, which are known to cause alterations in the levels of intestinal cyclic GMP and cyclic AMP, respectively, were used in the control experiments. The amounts of toxins administered to the mice are indicated in Table 1. Mouse intestines that received STs were removed for analysis after 3 h of incubation, while those that received CT were left for 8 h. STI and CT caused marked increases in intestinal cyclic GMP and cyclic AMP levels, respectively (Table 1). In contrast, in both suckling mice and adult mice, the levels of cyclic GMP and cyclic AMP in the intestines were not significantly altered by treatment with STII.

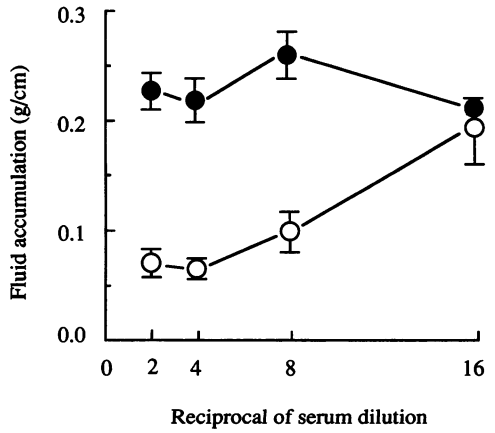

FIG. 4. Neutralization of STII activity by anti-STII serum. A volume of purified STII solution (5.6 μg/ml) was incubated with an equal volume of diluted anti-STII serum (○) or normal serum (●) as described in the text. After incubation, 0.18 ml of each solution was mixed with 0.02 ml of aprotinin solution (25,000 U/ml). A 0.2-ml quantity of the mixture was injected into a mouse intestinal loop, and the ratio of the weight of the loop (in grams) to the length of the loop (in centimeters) was determined after 3 h. Values represent means ± standard errors for five determinations.

Release of PGE_2 into intestinal lumens. It is known that prostaglandins induce duodenal and jejunal secretions of water and electrolytes in humans (32). Recently, several experiments on the mechanism of action of CT were done, and it was clear that (i) the amounts of prostaglandins in rabbit intestinal tissues increased with CT treatment and the increased prostaglandin amounts were released into the rabbit intestinal lumens and (ii) the effects of CT on intestinal secretion were inhibited by drugs, such as indomethacin and aspirin, that inhibit prostaglandin formation (6, 9, 25). These results implicate prostaglandins in the mechanism of action of CT.

To determine whether prostaglandins were also involved in the mechanism of action of STII, we performed two experiments. In the first, the level of PGE_2 in the fluid accumulated in mouse intestinal lumens by treatment with STII was examined. In the second, an examination was made of the effects of indomethacin and aspirin on the action of STII.

The level of PGE_2 in the fluid accumulated in mouse intestinal loops after exposure to STII was determined as described in Materials and Methods. As already mentioned, it has been demonstrated that CT causes an elevation in the amount of PGE_2 in the intestinal fluid of rabbits. For control experiments, we injected CT into mouse intestinal loops and examined the level of PGE_2 in the accumulated intestinal fluid. The level of PGE_2 increased (Fig. 5A), indicating that mice are appropriate experimental animals for examining the involvement of prostaglandins in intestinal fluid secretion. The level of PGE_2 in the intestinal fluid accumulated as a result of the action of STII also increased (Fig. 5B).

Treatment with drugs. Mice were given intraperitoneal injections of indomethacin or aspirin as described in Materials and Methods. Three hours later, 0.2 ml of a solution containing 500 ng of STII was injected into intestinal loops.

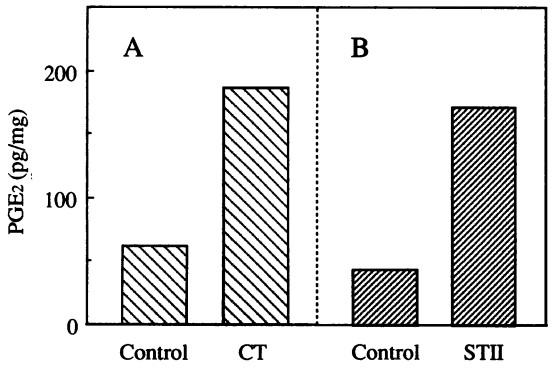

FIG. 5. Release of PGE_2 into intestinal fluid after exposure to STII and CT. Mouse intestinal loops were incubated for 8 h with 1 μg of CT (A) and for 3 h with 2 μg of STII (B). The PGE_2 content and the protein content in the accumulated fluid were determined as described in the text. Values represent means for five determinations.

The responses of the mice to STII are shown in Fig. 6. Treatment with either aspirin or indomethacin significantly reduced the secretion caused by STII, compared with that in control mice receiving toxin alone.

Histopathological examination. Light micrographs of the jejunum exposed to 1 μg of STII for 3 h are shown in Fig. 7. A dilation of the capillaries in the submucosa and a decrease in the thickness of the lamina propria were observed. There was no evidence of cellular damage or inflammation.

DISCUSSION

In this article, we describe the biological properties of purified STII. STII was reported to be active in the intestinal loop assay of weaned pigs but negative in those of other species (2, 14). It became clear that STII is sensitive to protease degradation and that the use of protease inhibitor to block intestinal protease activity causes an intestinal response to STII in rat intestinal loop and suckling mouse assays (40). In this study, we used mice, which are easier to work with as experimental animals than rats. Mouse intestinal loops also responded to STII in the presence of a protease inhibitor (Fig. 1). Subsequently, we found that loops created from washed intestines were more sensitive to STII than loops created from unwashed intestines (Fig. 1). Intestinal proteases are secreted from the pancreas to aid digestion in the intestines, so it is likely that removal of the intestinal contents by washing brought about a reduction in the secretion of proteases into the intestinal lumen. Also, proteases that already had been secreted into the intestines were likely removed by washing. Therefore, we predicted that washing of the intestines would make them more responsive to STII.

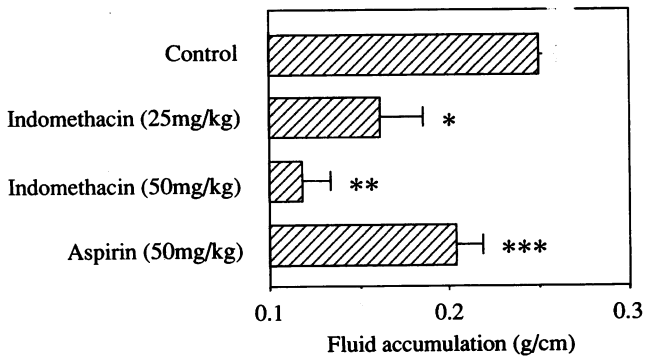

FIG. 6. Effects of indomethacin and aspirin on the intestinal fluid response to STII. The indicated amounts of indomethacin and aspirin were administered to mice by intraperitoneal injections with 10 mM Tris-HCl containing 0.9% NaCl as the diluent. Control mice were given only the diluent. Three hours later, the mice were anesthetized, intestinal loops were created, and 0.2 ml of a solution containing STII (2.5 μg/ml) and aprotinin (2,500 U/ml) was injected into them. The fluid accumulated in each loop was determined 3 h after the injection as described in the text. Values represent means ± standard errors for 7 to 10 determinations. Significance (versus the control): *, $P < 0.04$; **, $P < 0.001$; ***, $P < 0.05$.

It has been shown that STII is a poor immunogen. Dubreuil et al. (5) injected STII emulsified in complete Freund's adjuvant into rabbits. Inoculations were repeated eight times with incomplete Freund's adjuvant. However, the antibody titer remained low despite several booster doses, and the serum could not neutralize STII activity. Lawrence et al. (16) reported the production of anti-STII serum by the injection of a fusion protein (OmpF–STb fragment–β-galactosidase) or a 19-amino-acid peptide. The serum reacted weakly at low dilutions with the enterotoxin in the culture supernatant. However, the serum cross-reacted with unknown agents in non-STII-producing strains, and it remained unclear whether antiserum possessing the ability to neutralize the activity of purified STII could be produced. However, the results in Fig. 4 clearly show that antibodies able to neutralize the activity of purified STII can be produced. The anti-STII neutralizing serum obtained did not neutralize either STI activity or CT activity, indicating that CT and STI do not share immunological determinants with STII. The serum obtained should be very useful for further studies on enterotoxins.

The implication of STII in human diarrheal disease caused by enteric strains of *E. coli* is not clearly elucidated, although it has been shown that some strains producing only STII are responsible for diarrheal disease in swine (17, 19). On the other hand, STI and CT are known to be contributing factors in diarrheal disease caused by strains of *E. coli* that produce these toxins (27). We compared the diarrheagenic capacity of STII with those of STI and CT by using the mouse intestinal loop assay (Fig. 3). The results suggested that STII has enough activity to function as a diarrheagenic agent. Lortie et al. reported the isolation of STII-producing strains from human stools (17). It is possible that STII plays a role not only in swine but also in human diarrheal disease.

To clarify the mode of action of STII, we investigated its activity over time and intracellular mediators. These characteristics of CT and STI were also investigated. It was reported that action over time in suckling hamsters occurred early for STI and late for CT (35). However, the action over time for STII was not examined extensively. We used the mouse intestinal loop assay to examine the actions over time of these toxins because mice are easier to work with than suckling hamsters. The actions over time for CT and STI in mouse intestinal loops resembled those in suckling hamsters; i.e., the action in mouse intestinal loops occurred early for STI and late for CT (Fig. 2). The action over time for STII was quite different from that for CT. The early action of STII was similar to that of STI, but the rate of decrease in the level of fluid accumulated was slower for STII than for STI. The fluid accumulated as a result of the action of STII was still present in the loops by 8 h, but the fluid accumulated as a result of the action of STI had almost disappeared by 8 h

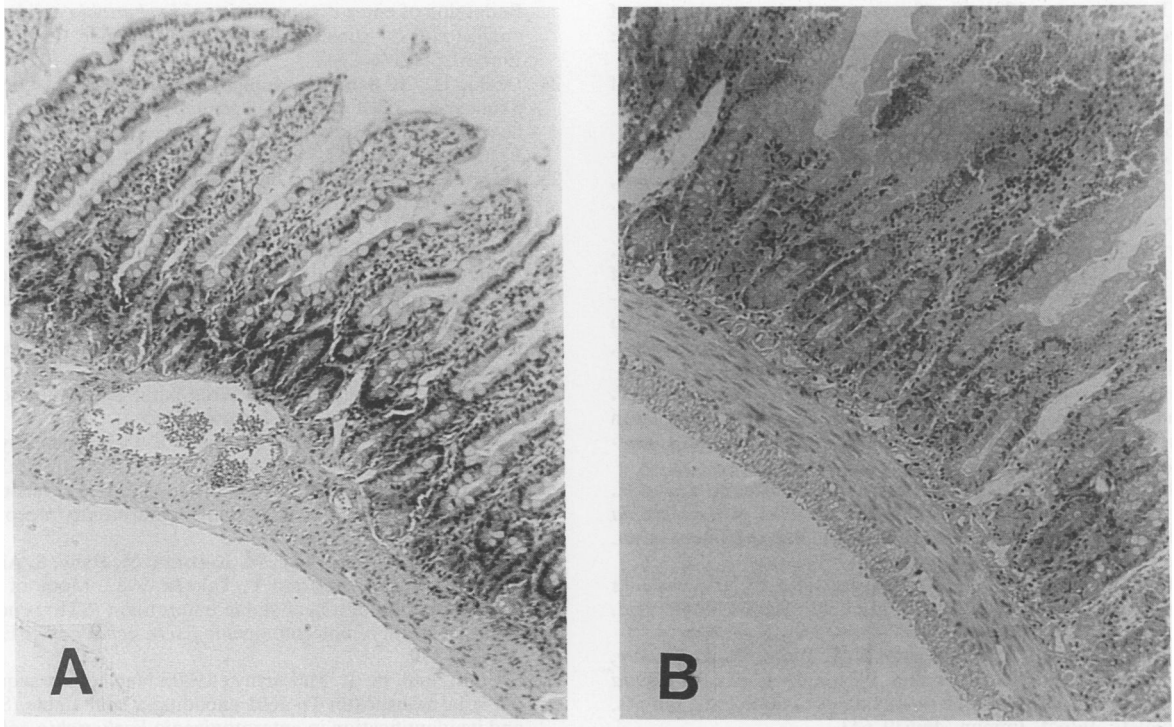

FIG. 7. Micropathology of mouse ileal mucosa exposed to STII. Mouse intestinal loops created as described in the text were injected with either 1 μg of purified STII (A) or saline (B). After 3 h of incubation, full-thickness sections were taken and immediately placed in 10% formalin.

(Fig. 2). The difference probably indicates that the fluid secretion caused by STII is mediated by a mechanism different from that of STI and CT.

STI and CT are known to elevate intracellular levels of cyclic GMP and cyclic AMP, respectively (26, 36). We examined the effect of purified STII on the intracellular levels of these cyclic nucleotides (Table 1). STII did not increase the levels of these cyclic nucleotides, demonstrating that STII induces secretion by a mechanism that is different from that of STI and CT.

It is known that PGE_1 and PGE_2 induce duodenal and jejunal secretions of water and electrolytes in humans and rabbits (13, 32). Peterson and Ochoa recently reported that CT injected into rabbit intestinal loops caused increases in the PGE_1 and PGE_2 contents of intestinal tissues and indicated that these prostaglandins may regulate water and electrolytes in cholera (25). They also found that analysis of the fluid in the loops yielded more reliable estimates of prostaglandin concentrations than did analysis of tissues, because of the rapid export of prostaglandins from cells after synthesis. We confirmed the work of Peterson and Ochoa (25) and observed similar results with STII in mouse intestinal loops (Fig. 5A). In addition, we found that PGE_2 levels also increased in mouse intestinal loops treated with STII (Fig. 5B). This result indicates that PGE_2 is also involved in the onset of diarrhea caused by STII.

It has been stated that prostaglandins change the blood volume in intestinal mucosal cells and that this change has a causal relationship to diarrhea (3, 13). In histopathological studies of intestines exposed to STII, increases in the blood volume resulting from dilation of the capillaries were observed (Fig. 7). Furthermore, aspirin and indomethacin, inhibitors of cyclooxygenase and therefore also of prostaglandin synthesis, significantly reduced the activity of STII (Fig. 6). These observations support the idea that PGE_2 is involved in the secretion of water induced by STII. More studies are in progress in our laboratory to further clarify the mechanism of action of STII.

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