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Involvement of 5-Hydroxytryptamine and Prostaglandin E_2 in the Intestinal Secretory Action of *Escherichia coli* Heat-Stable Enterotoxin B

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The intestinal secretory action of *Escherichia coli* heat-stable enterotoxin B (STb) is poorly defined. Previous work indicates that STb causes loss of intestinal fluid and electrolytes by a mechanism independent of elevated levels of cyclic nucleotides, the hallmark of other *E. coli* cytotonic enterotoxins. In the work described in this report, we observed that treatment of ligated rat intestinal loops with purified STb of *E. coli* resulted in a dose-dependent rise in intestinal secretion concomitant with dose-related increases in levels of serotonin (5-hydroxytryptamine [5-HT]) and prostaglandin E_2 (PGE_2). Treatment of rats with the 5-HT_2 receptor antagonist ketanserin prior to STb challenge resulted in significant ($P < 0.05$) reduction in intestinal secretion. Blockage of 5-HT_2 receptors with ketanserin also reduced ($P < 0.05$) the level of PGE_2 observed following STb treatment, indicating that at least a portion of the PGE_2 was formed in response to 5-HT_2 receptor stimulation. In a similar fashion, indomethacin, an inhibitor of cyclooxygenase activity, significantly reduced the level of secretion ($P < 0.05$) observed following STb treatment yet had no effect on 5-HT levels. Treatment of rats with both ketanserin and indomethacin further reduced STb-mediated secretion to a level not attained by either drug alone. Taken together, our data suggest that secretion due to STb involves both 5-HT and PGE_2 as intestinal secretagogues. Furthermore, PGE_2 formation appears to arise through both 5-HT-dependent and 5-HT-independent pathways.

The heat-stable enterotoxins of *Escherichia coli* are peptides which alter normal gut physiology, resulting in intestinal secretion. Currently, two heat-stable enterotoxins, STa and STb, are recognized. STa is an 18- or 19-amino-acid protease-resistant peptide that contains three disulfide bonds and has an acidic pI (9, 28, 29). STa binds to intestinal guanylate cyclase C, causing enzyme activation and a subsequent elevation of the concentration of mucosal cyclic GMP (cGMP) (13, 17, 27). Secretion is thought to be a consequence of an elevated concentration of cGMP, which couples to a net loss of chloride ions and water into the intestinal lumen. In contrast to STa, STb is a 48-amino-acid basic peptide that contains two disulfide bonds and is sensitive to trypsin-like proteolysis (11, 15, 34). Early studies indicate that unlike STa and the cholera toxin (CT)-like *E. coli* heat-labile enterotoxins, STb-mediated secretion occurs in the absence of elevated levels of cyclic nucleotides (21, 32). Although the mechanism of action of STb is poorly understood, STb was recently shown to elevate intestinal prostaglandin E_2 (PGE_2) levels (20). PGE_2, a known intestinal secretagogue, has also been implicated in CT-mediated secretion (4, 25). In addition to PGE_2, CT-mediated secretion appears to involve the release of 5-hydroxytryptamine (5-HT) (3, 4). In the gut, PGE_2 and 5-HT promote intestinal secretion by apparently independent mechanisms, yet the release of 5-HT and the synthesis of PGE_2 may be coupled events in the gut.

Since a previous report suggested a potential role for PGE_2 in the mechanism of STb action (19), we investigated the involvement of 5-HT in the mechanism of STb intestinal secretion and its potential involvement in the pathway leading to PGE_2 formation. Our results indicate that 5-HT and PGE_2 are involved in STb-mediated secretion.

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MATERIALS AND METHODS

Toxin preparation. STb was purified by a modification of a previously reported method (11). Briefly, *E. coli* 1790 (obtained from Shannon C. Whipp, USDA Agricultural Research Services, National Animal Disease Center, Ames, Iowa) harboring pPD21K, a kanamycin-resistant derivative of plasmid pPD21 (30) used as a source of the STb gene, was grown at 37°C with vigorous aeration in 24 liters of M9 medium (23) containing 0.2% glucose and kanamycin (50 μg/ml). After 18 h the bacterial cells were removed by filtration at room temperature through a 0.1-μm-pore-size hollow fiber cartridge attached to a DC-10 preparative ultrafiltration device (Amicon, Inc., Beverly, Mass.). High-molecular-mass material was removed from the filtrate by passage through a 100-kDa exclusion spiral membrane cartridge (Amicon). The filtrate fraction (about 24 liters) was concentrated over a 3-kDa exclusion spiral membrane cartridge (Amicon) to a final volume of approximately 1 liter. The concentrated filtrate was then pumped at a flow rate of 30 ml/min onto a DeltaPak C_4 RCM preparative (25 by 100 mm) high-pressure liquid chromatography (HPLC) column (Waters Division, Millipore Corp., Milford, Mass.) by using a model 2350 preparative HPLC pump and gradient programmer (ISCO, Lincoln, Neb.). Bound material was eluted from the column with a step gradient of methanol containing 0.05% trifluoroacetic acid (TFA). The eluate was monitored at 220 nm with a V4 variable-wavelength monitor (ISCO); STb eluted at 60% methanol–0.05% TFA. Fractions containing STb were subjected to rotary evaporation to remove the methanol followed by solvent exchange over a Fast Desalt column (Pharmacia, Piscataway, N.J.) equilibrated in 0.05 M sodium phosphate buffer (pH 6.5). The sample was then fractionated by cation-exchange fast protein liquid chromatography (FPLC; Pharmacia) on a Mono-S column equilibrated in the same buffer. Material which bound to the Mono-S column was eluted with a linear gradient of 0 to 1 M NaCl in phosphate buffer (pH 6.5). STb eluted as a single peak (monitored at 226 nm) at approximately 0.3 M NaCl. Aliquots of the STb-containing peak were analyzed for purity by reverse-phase FPLC on a PepRPC 15 column (Pharmacia) equilibrated in water containing 0.05% TFA and developed with a linear gradient of 0 to 100% acetonitrile containing 0.05% TFA. Pure STb eluted as a single sharp peak at approximately 60% acetonitrile. The material was further analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (12.5% T, 6% C) in tricine buffer as described by Schägger and von Jagow (26) and automated Edman degradation with a model 470 automated sequencer (Applied Biosystems, Foster City, Calif.).

Rat ligated intestinal loop assay. Rat ligated intestinal loop assays were performed as described by Whipp (35). Female Sprague-Dawley rats (weighing 125 to 150 g each) were anesthetized with an intraperitoneal injection of Nembutal (60 mg/kg). The small intestine was exteriorized through a midline incision in the abdomen and flushed with 10 ml of a phosphate-buffered saline (PBS) solution containing 1 mg of soybean trypsin inhibitor (Sigma) per ml. Intestinal segments, 5 to 6 cm in length, were ligated with suture material starting 2 cm from the ligament of Treitz and proceeding posterior with 1-cm interloops connecting the test sections. Intestinal ligated loops were given injections of either 100 μl of STb in PBS-trypsin inhibitor solution or PBS-trypsin inhibitor solution alone (control). The intestine was then returned to the abdominal cavity, which was closed with clamps. After 90 min, the intestinal loops were emptied and fluid accumulation was recorded as volume/loop length (microliters per centimeter). Loops containing less than 500 μl of fluid were rinsed with PBS for a total collected volume of 500 μl.

Identification of 5-HT in intestinal fluid. For 5-HT determinations, fluid from each loop was mixed with an equal volume of 0.2% EDTA and 0.2 M perchloric acid, centrifuged at 14,000 × *g* for 5 min, and stored at −80°C until analyzed. Samples were fractionated by C_{18} reverse-phase HPLC as described by Morier-Teissier and Rips (24). Briefly, aliquots of the acidified loop contents were injected onto a Dynamax C_{18} reverse-phase column (Rainin Instruments) in a mobile phase consisting of 0.1 M KH_2PO_4 (pH 4.1), 0.1 mM EDTA, 5 mM heptane sulfonic acid (PIC B7; Waters Corp., Milford, Mass.), and 20% (vol/vol) methanol. Samples were developed by isocratic separation in the mobile phase; the effluent was monitored with a model LC-4B electrochemical detector (BAS Instruments, Indianapolis, Ind.). Known quantities of 5-HT run in duplicate were used to generate a standard curve. The identity of 5-HT was confirmed by HPLC/(atmospheric pressure chemical ionization) mass spectrometry/mass spectrometry [HPLC/(APCI) MS/MS]. HPLC/(APCI) MS/MS analysis was performed with a VG Quattro Tandem Mass Spectrometer connected to an HP 1090 liquid chromatograph via a heated pneumatic nebulizer. The serotonin-containing samples were run on a Spherisorb ODS-2 4.3-mm reverse-phase column and eluted with a gradient mobile phase of acetonitrile with 0.1% TFA. The eluate from the liquid chromatograph was split, so that 50% flowed to a UV detector operating at 275 nm and 50% flowed to the mass spectrometer. Corresponding 5-HT standards and HPLC-purified intestinal samples (see above) showed identical UV profiles. For 5-HT detection, the mass filter was fixed at 177 Da to transmit the protonated molecular ions of serotonin. These ions were then directed to a collision cell, where they underwent collision-induced dissociation with a collision energy of 25 eV in neutral argon gas (0.1 Pa) with a dwell time of 0.2 s per ion. The resulting daughter ions were then monitored by the second mass filter at 177+ to 160+ Da.

Identification of PGE_2 in intestinal fluid. Intestinal loop samples were prepared for PGE_2 analysis by addition of 100 μl of 1 N HCl with 5% trichloroacetic acid to 500 μl of loop fluid. Samples were centrifuged (14,000 × *g*), and the supernatant fractions were each mixed with 3 ml of water-saturated ether. Following a 1-min agitation, the aqueous phase was mixed with 3 ml of ethyl acetate–isopropanol–0.2 N HCl solution (3:3:1, vol/vol/vol) and vortexed twice for 15 s each. Finally, 2 ml of ethyl acetate and 3 ml of water were added to each sample. Following centrifugation (5 min at 5,000 × *g*), the organic phase was collected and dried in a vacuum concentrator. The PGE_2 concentration in each sample was determined with a radioimmunoassay kit (Prostaglandin E [^{125}I] assay system; Amersham Corp., Arlington Heights, Ill.) and recorded as amount of PGE_2 per loop length (picograms per centimeter).

Pharmacological agents and other chemicals. Ketanserin, was obtained from Research Biochemicals Inc., Natick, Mass. Indomethacin, 5-HT (oxalate salt), and trypsin inhibitor were purchased from Sigma Chemical Co., St. Louis, Mo. All other chemicals were of the highest purity available and were obtained from common suppliers.

RESULTS

Effect of STb on rat intestinal secretion. STb caused a dose-dependent increase in intestinal secretion following the 90-min incubation period (Fig. 1). In contrast to these results, control loops receiving PBS were nearly empty (≤10 μl/cm) following the incubation period. A consistently observed result was that the position where the loop was placed within the intestine influenced the volume of STb-mediated secretion detected. To test the effect of loop position on secretion, 1 μg of STb was injected into each of five 6-cm loops in defined positions relative to the ligament of Treitz. Following incubation, it was observed that intestinal segments near the ligament of Treitz exhibited nearly twofold less secretion in response to STb than did more distal loops (data not shown). Similar results were previously reported by Whipp (35). As a result of this observation, loop position was closely monitored when dose-response experiments were set up and also when loop contents were to be analyzed.

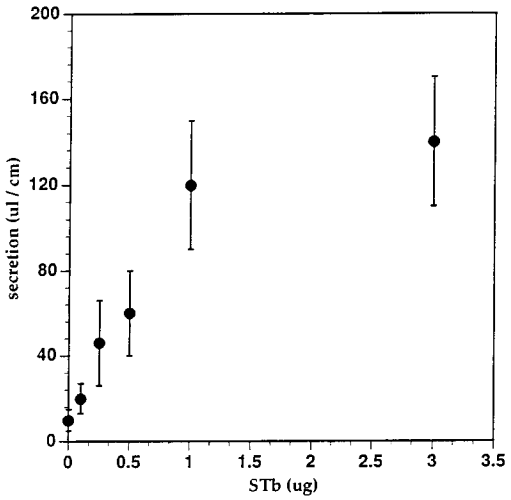

FIG. 1. Dose-dependent STb-mediated secretion. Rat ligated intestinal loops were treated with doses of STb in PBS containing trypsin inhibitor and PBS alone containing trypsin inhibitor (control), as described in Materials and Methods. Each datum point represents the mean result for three loops from two different rats ± standard error of the mean.

Detection of 5-HT in STb-treated intestinal tissue. A previous report demonstrating the appearance of PGE_2 in the gut of mice treated with STb (19) and reports suggesting the involvement of PGE_2 and 5-HT in CT-mediated secretion led us to examine the presence of 5-HT in STb-treated intestinal fluid. HPLC analysis (isocratic C_{18} reverse-phase system) of intestinal fluid obtained following STb treatment revealed a peak at 16 min in the HPLC profile, which comigrated with a 5-HT standard (Fig. 2A). Samples containing the peak at 16 min generated by C_{18} reverse-phase HPLC were collected and confirmed to be 5-HT by HPLC/(APCI) MS/MS (Fig. 3). To verify that 5-HT was the only electrochemically detected compound present in the peak eluting in the HPLC profile, an equivalent quantity of 5-HT, as determined in the sample shown in Fig. 2A (3 ng), was added to the sample, which was then rerun on the C_{18} reverse-phase column. The results demonstrated the predicted peak height doubling shown in Fig. 2B. An identical analysis was performed with a C_8 reverse-phase column (4.6- by 250-mm Microsorb C_8 column [Rainin Instruments]); standard and gut loop 5-HT coeluted as a single sharp peak at approximately 8 min, thus verifying that 5-HT was the only electrochemically detected compound in the peak.

The effect of STb action on intestinal secretion and 5-HT production is shown in Fig. 4. In addition to dose-dependent intestinal secretion, STb caused a dose-related rise in the level of 5-HT (Fig. 4). Control loops were nearly empty (≤10 μl/cm), and washings of the gut loop contained minimal amounts of 5-HT (Fig. 4). Doses of STb greater than 1.0 μg, which failed to yield higher levels of secretion (Fig. 1), did not induce the release of significantly higher levels of 5-HT (data not shown).

To investigate the potential relationship between 5-HT and STb-mediated secretion, we tested the effect of treating rats with the 5-HT_2 receptor antagonist ketanserin prior to STb challenge. If STb-mediated intestinal secretion involves 5-HT, blocking 5-HT receptors in the gut should reduce the amount of fluid recovered in STb-treated intestinal loops. Since 5-HT_2 receptors have been shown to mediate fluid secretion in the gut

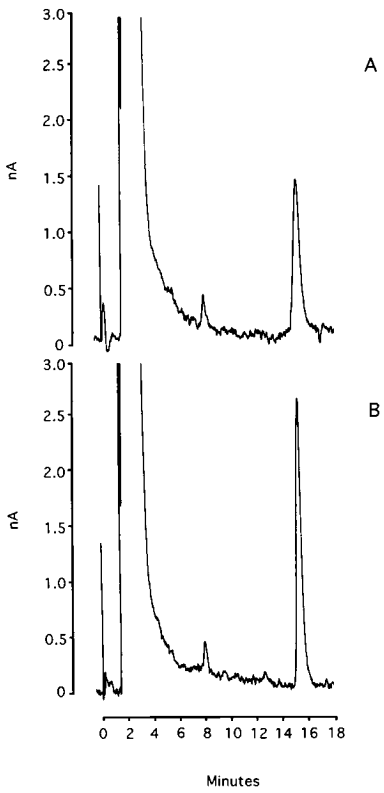

FIG. 2. HPLC analysis of STb-induced intestinal fluid. An STb-treated intestinal loop sample containing 3 ng of 5-HT as determined by standard curve (A) and the same sample with an additional 3 ng of standard 5-HT added (B) were separated as described in the text. The peak at 16 min was determined by MS to be 5-HT. Full-scale deflection of the electrochemical detector was 5 nA. Panel B illustrates the doubling of the 5-HT peak height by the addition of an equivalent quantity of commercial standard 5-HT.

in response to 5-HT (2, 4, 18), ketanserin, a 5-HT_2-specific receptor antagonist, was tested for the ability to block STb action. Basal secretory levels ($\leq$10 μl/cm) were observed in rats challenged with 0.25 μg of STb per loop and pretreated with 17.5 μg of ketanserin per loop (Fig. 5). Ketanserin also significantly inhibited secretion when loops were challenged with 0.5 and 1.0 μg of STb, resulting in 66.7 and 57.1% inhibition, respectively (Fig. 5).

Role of PGE_2 in STb-mediated secretion. A previous study indicated that PGE_2 was present in the gut of STb-treated mice (20). We therefore examined the effect of STb on PGE_2 levels in rat intestinal loops and its association with subsequent fluid accumulation. Our results (Fig. 6) demonstrated that STb caused a dose-dependent rise in luminal PGE_2 levels following STb treatment. Secretion also increased in a dose-related manner.

The relationship between PGE_2 formation and intestinal secretion due to STb was tested by assessing the effect of indomethacin, a PG synthesis inhibitor, on STb-mediated secretion. Rats were given intraperitoneal injections of indomethacin (50 mg/kg) 3 h prior to challenge with STb. Following incubation with 1 μg of STb, control loops contained 500 μl of fluid per intestinal loop ($n = 6$) whereas indomethacin-treated rats contained 275 $\pm$ 5 μl per loop ($n = 6$); i.e., a 45% inhibition of secretion was observed (Fig. 5, right-hand bar). These data are similar to those reported for indomethacin treatment of STb-challenged mice (19).

Effect of inhibitors on 5-HT and PGE_2 release. Since 5-HT_2 receptors have been shown to modulate arachidonate metabolism in neuronal tissue (6, 12, 20), we examined the effect of ketanserin on PGE_2 synthesis. Loops pretreated with ketanserin (17.5 μg per loop) and challenged with 1.0 μg of STb showed a significant ($P < 0.05$), 27% decrease in PGE_2 levels. Increasing the ketanserin dose caused a slight (37%) but insignificant further reduction in PGE_2 levels compared with the 27% decrease observed with the lower ketanserin concentration. Intestinal loop fluid from rats treated with 50 mg of indomethacin per kg was assayed for the presence of 5-HT. Pretreatment with indomethacin resulted in no significant change in 5-HT levels (data not shown). Taken together, these results indicated that while 5-HT was at least in part responsible for PGE_2 release into the intestinal lumen. PGE_2 had no apparent effect on 5-HT release.

Combined effect of ketanserin and indomethacin on STb-mediated secretion. Pretreatment of rat intestinal loops with ketanserin completely inhibited intestinal secretion due to a low dose of STb (0.25 μg per loop [Fig. 5]); however, higher doses of STb could not be completely inhibited by ketanserin. Further reduction of secretion was obtained with higher concentrations of ketanserin (33 μg per loop), but the increased inhibition was insignificant and incomplete (results not shown). Since secretion due to STb may involve both 5-HT and PGE_2 and since a significant portion of the STb-related PGE_2 formation was not attributed to luminal release of 5-HT, we tested the combined ability of ketanserin and indomethacin to inhibit STb secretion. As with ketanserin alone, rats pretreated with both ketanserin and indomethacin and challenged with 0.25 μg of STb showed complete inhibition of toxin-mediated secretion (Fig. 5). At a higher dose of STb (1 μg), a small but significant ($P < 0.05$) further reduction in the level of secretion observed following ketanserin treatment was noted when rats were given ketanserin and indomethacin prior to STb (Fig. 5). The reduction in STb-mediated secretion was greater than the inhibition of secretion achieved by either inhibitor alone, suggesting the potential involvement of both 5-HT and PGE_2 in STb action.

DISCUSSION

Previous reports indicate that STb causes intestinal secretion in the absence of elevated levels of cyclic nucleotides (21, 32), the hallmark of other *E. coli* cytotonic enterotoxins. Ussing chamber studies indicated that STb induced short-circuit current across stripped porcine mucosa when introduced on the mucosal (but not the serosal) side of the mounted tissue (32). The secreted anion responsible for the net current flow in Ussing chamber studies was thought to be bicarbonate, not the chloride secretion due to STa (33). Recent reports have provided new insights into STb action which served as the initiative for the study reported here. Hitotsubashi et al. (19) reported that STb caused an increase in the PGE_2 level in the gut of toxin-treated mice. Similarly, PGE_2 has been implicated in the intestinal secretory action of CT (4, 25). Involvement of 5-HT, another intestinal secretagogue, has also been reported to occur in secretion due to CT (4). Recently, Dreyfus et al. (10) reported the ability of STb to elevate cytosolic calcium levels in cultured and primary cells. Collectively, the properties attributed to STb, including release of PGE_2, secretory action independent of cyclic nucleotide elevation, and calcium ion elevation, indicated the potential involvement of serotonin (5-HT) in the STb secretory response. Elevated 5-HT levels are known to mediate intestinal water and electrolyte transport without alterations in adenylate cyclase activity, cyclic nucleotide phosphodiesterase, or Na^+,K^+-ATPase (8, 22). 5-HT-mediated changes in electrolyte transport are calcium depen-

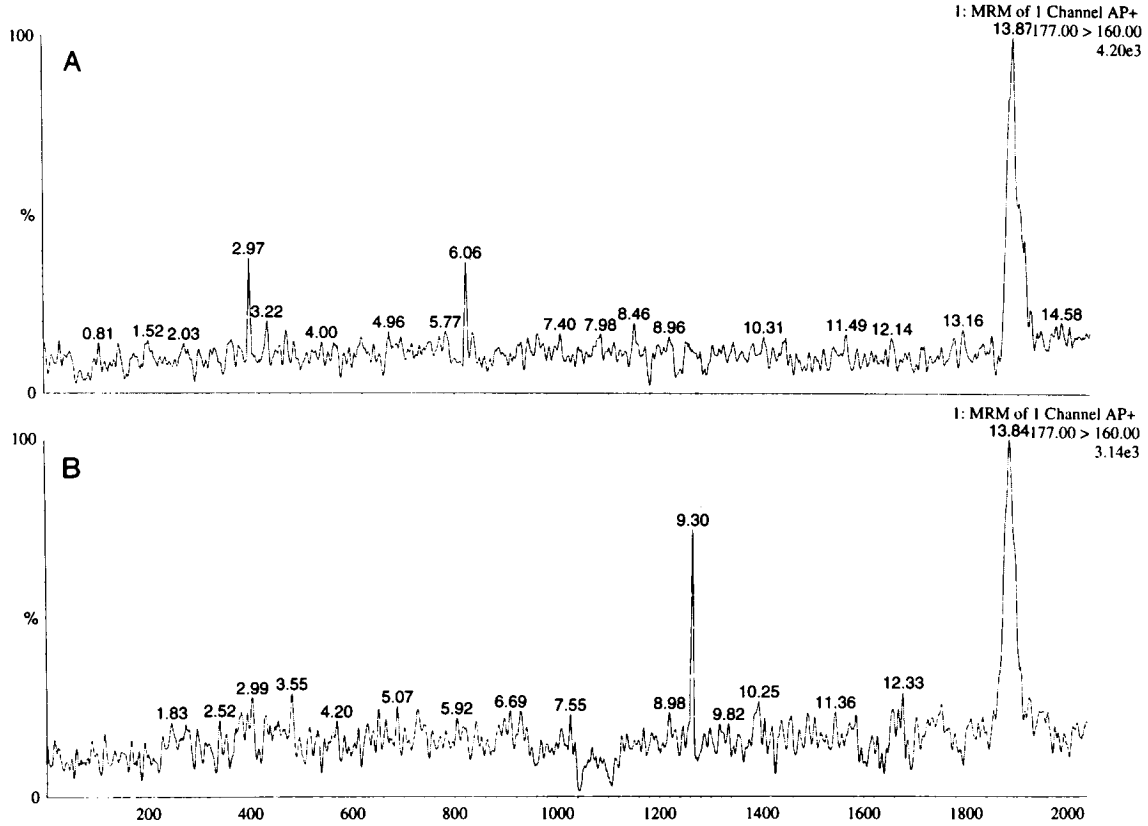

FIG. 3. MS of intestinal 5-HT. The daughter ion mass spectrum of standard 5-HT (A) and the corresponding spectrum of intestinal fluid from an STb-treated rat (B) are shown. Following on-line C_{18} HPLC separation of standard or intestinal sample material, the APCI-generated cation was monitored at 177 Da ($M + H^+$). Following fragmentation by collision-induced dissociation, daughter ion formation (177^+ to 160^+) was monitored at 160 Da. Stable daughter ion formation was first determined for purified standard serotonin, and the determination was repeated with experimental standards. The *x* coordinate is HPLC retention time in minutes. The *y* coordinate is percent full-scale deflection.

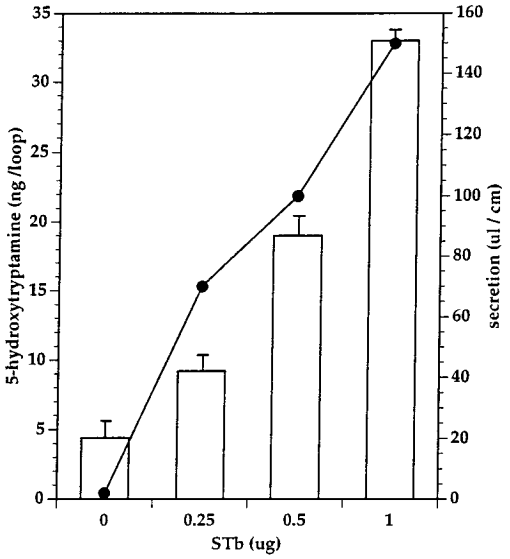

FIG. 4. Effect of STb on 5-HT release and intestinal secretion. Datum points represent mean secretion from two rats (six loops) treated with STb for 90 min (solid circles and line graph). Bars represent the amount of 5-HT in the same rats ± standard error of the mean.

dent in that the increase in short-circuit current induced by 5-HT was diminished in calcium-free medium and mimicked by the calcium ionophore A23187 (1, 5). The calcium dependence of 5-HT-mediated secretion was postulated to involve an increase in $[Ca^{2+}]_i$ (1, 5, 7).

The observed similarities between STb action and 5-HT release and activity led us to investigate the possible role of 5-HT in STb-mediated secretion. Our results indicate a role for 5-HT in STb-mediated secretion. Our results also confirm those of Hitotsubashi et al. (19) by indicating a role for PGE_2 in secretion due to STb; in addition, our data reported here suggest that PGE_2 formation is in part due to the action of 5-HT. 5-HT may lead to PGE_2 synthesis through $5\text{-}HT_2$ receptor stimulation coupled to activation of phosphoinositide turnover (6, 20) and/or activation of phospholipase A_2 (12). It is also clear, however, that STb action results in a 5-HT-independent biosynthesis of PGE_2, since ketanserin, a $5\text{-}HT_2$ receptor antagonist, only partially blocked PGE_2 formation in response to STb. The failure of ketanserin to completely block PGE_2 synthesis suggests that STb may act directly to promote arachidonate release and metabolism. Certainly, the ability to elevate internal calcium levels through a G-protein-coupled calcium ion channel (10) could also lead to activation of the phosphatidylinositol cascade, resulting in prostanoid synthesis. The ability of STb to mediate arachidonic acid metabolism in rat intestinal tissue in vitro is currently under investigation.

The potential involvement of 5-HT in the mechanism of STb secretion is of particular interest, since until now, only PGE_2

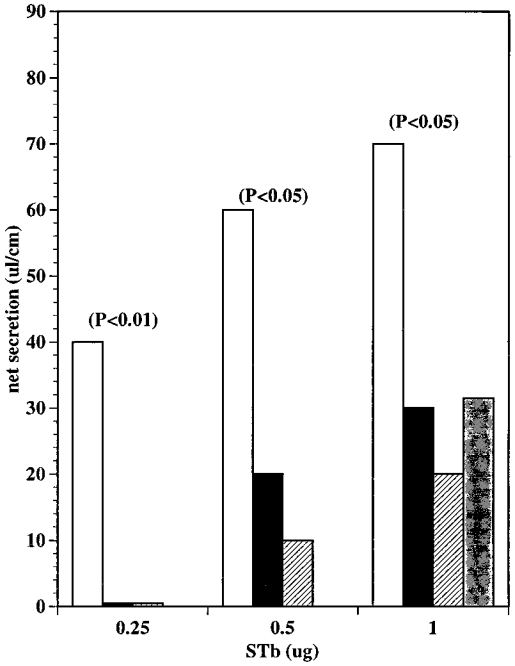

FIG. 5. Effects of ketanserin and indomethacin on STb-mediated intestinal secretion. Intestinal loops were treated with 17.5 μg of ketanserin per loop (hatched bars) 15 min prior to injection of STb, resulting in 100, 66.7, and 57.1% inhibition of secretion. Solid bars represent rats treated for 3 h with indomethacin (50 mg/kg) and 15 min with ketanserin. Rats treated with both inhibitors show 100, 83.3, and 71.4% inhibition of secretion when challenged with 0.25, 0.5, and 1.0 μg of STb, respectively. Open bars represent net secretion for 0.25, 0.5, and 1.0 μg of STb. The gray bar at the far right represents inhibition of secretion (45%) by 50 mg of indomethacin per kg given intraperitoneally 3 h prior to challenge with 1 μg of STb. Standard error of the mean was <1% for all samples. The significance value, determined by the *t* test, is for STb secretion relative to inhibition by ketanserin. Values for ketanserin plus indomethacin were of equivalent or greater significance.

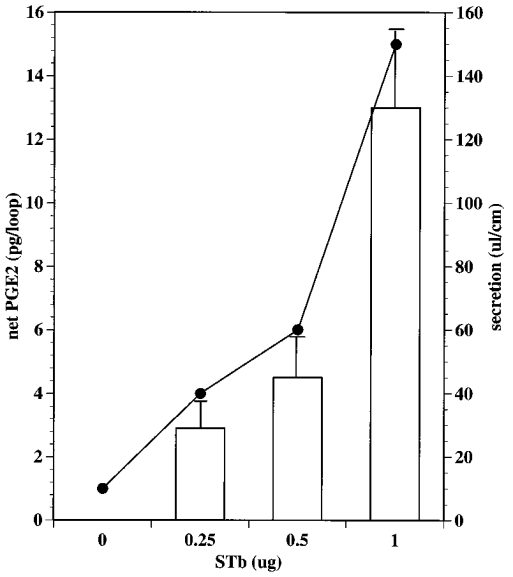

FIG. 6. Effects of STb on PGE_2 release and intestinal secretion. The data represent the mean amount of PGE_2 minus the control value (11.25 pg per loop) (bars) and intestinal secretion (solid circles and line graph). Each datum point represents mean secretion from two rats (six loops per datum point).

has been implicated as an STb-induced secretagogue. Intestinal 5-HT is located primarily in lumen-exposed enterochromaffin cells and neurons of the myenteric plexus (16). Although the role of 5-HT in intestinal secretion and motility is well characterized, elucidating the specific 5-HT receptor type through which gut secretory mechanisms couple has been difficult. Factors contributing to the complexity of the issue include (i) the number of 5-HT receptor subtypes present (at least five different 5-HT receptors, including 5-HT_{1A}, 5-HT_{1P}, 5-HT_2, 5-HT_3, and 5-HT_4, are known to exist in the gut) (16), (ii) the differential distribution of receptor subtypes throughout the intestine, and (iii) the nonselectivity of most 5-HT receptor antagonists (31). Release of 5-HT into the lumen of the intestine by STb suggests that 5-HT_2, 5-HT_3, and/or 5-HT_4 receptors may be involved in the secretory process, since these receptors are implicated in mediating intestinal secretion in rats given luminal doses of 5-HT in vivo (18). Support for this notion may be derived from the observed inhibition of STb-mediated secretion following ketanserin treatment reported here and the observation that 5-HT_3 receptor antagonists also reduce STb secretion (18a). In addition, the involvement of multiple 5-HT receptors in STb-induced secretion may account for the lack of complete inhibition of secretion by ketanserin at higher STb doses. Although these putative mechanisms address the appearance and significance only of mucosal 5-HT, the STb-mediated effect on enterochromaffin cells probably results in serosal release of 5-HT as well. The release of 5-HT into serosal tissue and the concomitant involvement of 5-HT receptors of the enteric nervous system may then couple directly to ion and water secretion (16). Experiments designed to delineate the events which trigger 5-HT release and subsequent action are in progress.

Elements shown here to be involved in the secretory mechanism of STb have also been suggested by Beubler et al. (3, 4) to explain CT-induced secretion. Dose-dependent rises in 5-HT and PGE_2 were observed in rats treated with CT; treatment of rats with ketanserin and indomethacin significantly reduced CT-mediated secretion. The inability of ketanserin and indomethacin to completely block CT secretion was attributed to secretory effects of elevated levels of cyclic AMP (cAMP), which had no apparent role in the CT-mediated elevation of 5-HT and PGE_2 (14). Peterson and Ochoa (25) also noted that CT-induced secretion in rabbits correlated better with PGE_2 levels than with cAMP levels. Although elevated 5-HT and PGE_2 levels are involved in intestinal secretion due to CT and STb, the mechanisms by which these two dissimilar toxins promote these events are presently unclear.

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