Serotonin stimulates endotoxin translocation via 5-HT\textsubscript{3} receptors in the rat ileum

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Yamada, Tadanori, Akio Inui, Naoki Hayashi, Masaki Fujimura, and Mineko Fujimiya. Serotonin stimulates endotoxin translocation via 5-HT\textsubscript{3} receptors in the rat ileum. Am J Physiol Gastrointest Liver Physiol 284: G782–G788, 2003; 10.1152/ajpgi.00376.2002.—Because few previous studies have investigated the mechanisms of endotoxin translocation induced by intestinal obstruction, we aimed to clarify whether or not serotonin [5-hydroxytryptamine (5-HT)], which is released from enterochromaffin (EC) cells, is responsible for alternations of the mucosal permeability to endotoxin and to identify the 5-HT receptor subtypes that mediate this action. FITC-labeled LPS (FITC-LPS) was injected into the ileum of rats, and the FITC-LPS level in the superior mesenteric vein was subsequently measured by using a fluorescence spectrophotometer. To measure the 5-HT release induced by high intraluminal pressure, ex vivo preparation of vascularity and luminally perfused rat ileum was used. Results demonstrated that elevated intraluminal pressure stimulates the translocation of FITC-LPS and the release of 5-HT from the EC cells into the intestinal lumen but not into the portal circulation. This FITC-LPS translocation, which was stimulated by exogenously applied 5-HT in the lumen and the jugular vein, was inhibited by 5-HT\textsubscript{3} receptor antagonist administration both intumally and intravenously. The stimulatory effect of elevated intraluminal pressure on the translocation of FITC-LPS was inhibited by the intraluminal and intravenous administration of 5-HT\textsubscript{3} receptor antagonist. These results suggest that 5-HT released from EC cells may be involved in the translocation of FITC-LPS induced by elevated intraluminal pressure and that this effect is mediated by 5-HT\textsubscript{3} receptors that may be located in the intestinal epithelium.

THE GASTROINTESTINAL TRACT contains numerous bacteria and endotoxins that are prevented from reaching the systemic circulation by the barrier function of the intestinal mucosa. However, this barrier is disrupted by various pathological insults, including hemorrhage, intestinal obstruction, immunosuppression, thermal injury, nonthermal trauma, sepsis, radiation injury, and endotoxemia (5, 15, 18, 20). Possible mechanisms responsible for these disruptions have been widely investigated. Exogenously applied bacterial endotoxin (LPS) induces hyperpermeability of the intestinal mucosa in both in vitro (19) and in vivo (24) models. In the in vitro experiments, administration of LPS to the basolateral surface of the cultured epithelial cells altered the constitution of intestinal, tight-junction-associated proteins and subsequently disrupted the barrier function, whereas LPS administered to the apical surface of the cells had no effect (19). In the in vivo experiments, systemic administration of LPS induced an increase in the intestinal permeability of humans (24). The intestinal permeability may be altered by a number of etiological factors, including mesenteric hypoperfusion, as demonstrated in endotoxic pigs (8) and hyperactivity of the inducible nitric oxide synthase of the intestinal mucosa, as demonstrated in Ussing chamber preparation of rat ileum (23). A number of previous studies have investigated the effects of biogenic amines, such as serotonin [5-hydroxytryptamine (5-HT)] and histamine, on endotoxin translocation. However, these studies focused on the effect of these amines on vascular permeability with little attention paid to the effect on epithelial permeability (4). Another study demonstrated that 5-HT and its analog inhibits bacterial translocation by stimulating intestinal motility and subsequently preventing bacterial overgrowth (28), 5-HT stimulates intestinal motility (22), mucosal electrolyte transport (21), and vascular permeability (4). However, the effect of 5-HT on the intestinal epithelium regarding the permeability of endotoxin has not been demonstrated.

Intestinal obstruction, which is a clinically important etiologic factor of endotoxin translocation, may result in septicemia and death (5, 15). Mechanisms responsible for the bacterial translocation induced by intestinal obstruction have not been clarified. It has been demonstrated that elevated intraluminal pressure stimulates the release of 5-HT from enterochromaffin (EC) cells in rat duodenum (10) and guinea pig ileum (3) in both physiological and morphological studies. The increased 5-HT release from EC cells induced by elevated intraluminal pressure may cause an increase in epithelial permeability and may then induce endotoxin translocation from the intestinal lumen to peritoneum. This indicates that serotonin, which is released from EC cells, may be involved in the translocation of endotoxin into the bloodstream.

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the blood circulation. We used an in vivo model of the rat ileum to investigate the effect of 5-HT on the mucosal permeability induced by high intraluminal pressure and to identify the 5-HT receptor subtype that mediates this action.

MATERIALS AND METHODS

Measurement of FITC-labeled LPS translocated from ileum to superior mesenteric vein. Male Wistar rats weighing 500–600 g were fasted overnight (16–18 h) before the experiment. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg Nembutal; Abbott Laboratories). The terminal ileum, measuring ~10 cm in length, was prepared for in vivo luminal perfusion. Luminal perfusion was performed through a cannula inserted into the proximal end of the segment, and 0.1 M PBS (pH 7.4) containing 50 μg/ml of FITC-LPS (from Escherichia coli, serotype 0111, B4; Sigma) was perfused at a speed of 0.5 ml/min and was drained from the distal end of the ileal segment. Blood samples were collected by using a heparinized catheter (0.5 mm ID, 0.8 mm OD, model SV 31; Natsume, Tokyo, Japan) inserted into the splenic vein with the tip placed in the superior mesenteric vein. Blood was collected in 0.2-ml samples for 45 min at 5-min intervals into ice-cold vials. Blood samples were centrifuged (3,000 rpm) for 5 min, and the plasma levels of FITC were measured by using a fluorescence spectrophotometer (model F-4500) at an excitation of 485 nm and an emission of 535 nm. After a 15-min equilibration period, the intraluminal pressure was raised by clamping the distal end of the ileal segment, and the clamp was released when the pressure reached 40 cmH2O. The pressure was maintained at 3 and 1 ml/min, respectively. After a 30-min equilibration period, the vascular and luminal effluents were collected for 30 min at 5-min intervals into ice-cold vials. At 18 min, the intraluminal pressure was raised by clamping the luminal effluent. The clamp was released when the pressure reached 40 cmH2O. In some experiments, chemicals were injected intraluminally or intravascularly as described below, and the FITC levels in the superior mesenteric vein were compared with those of the nontreated controls. In some experiments, chemicals were injected intraluminally or intravascularly as described below, and the FITC levels in the superior mesenteric vein were compared with those of the saline-injected controls.

Luminal injection was performed through a cannula inserted into the ileal segment, and vascular injection was performed through a catheter inserted into the jugular vein. 5-HT (Sigma), at a concentration of 10, 1.0, or 0.1 μmol·kg⁻¹·min⁻¹, was injected into the lumen for 15–35 min. 5-HT at a concentration of 0.1 or 0.01 μmol·kg⁻¹·min⁻¹ was injected into the jugular vein for 15–35 min. The luminal injection of 5-HT (10 μmol·kg⁻¹·min⁻¹) and vascular injection of 5-HT (0.1 μmol·kg⁻¹·min⁻¹) was combined with the luminal (10 μg·kg⁻¹·min⁻¹) or vascular (1 μg·kg⁻¹·min⁻¹) injection of methysergide, a 5-HT₁/5-HT₂ receptor dual antagonist (San- doz Pharmaceutical, Basle, Switzerland), the luminal (100 μg·kg⁻¹·min⁻¹) or vascular (10 μg·kg⁻¹·min⁻¹) injection of ketanserin, a 5-HT₂ receptor antagonist (Janssen Pharmaceutica, Beerse, Belgium), the luminal (1 μg·kg⁻¹·min⁻¹) or vascular (0.1 μg·kg⁻¹·min⁻¹) injection of azasetron, a 5-HT₃ receptor antagonist (Yoshitomi Pharmaceutical), or the luminal (1 μg·kg⁻¹·min⁻¹) or vascular (0.1 μg·kg⁻¹·min⁻¹) injection of SB-204070, a 5-HT₄ receptor antagonist (Glaxo- SmithKline, London, UK). In these experiments, a 5-HT receptor antagonist was administered for 10–35 min. In some experiments, the luminal (1 μg·kg⁻¹·min⁻¹) or vascular (0.1 μg·kg⁻¹·min⁻¹) injection of azasetron was performed for between 10 and 35 min, and the intraluminal pressure was raised to 40 mmH2O during this time. Instead of the above chemicals, saline was injected into the controls. The dose of each 5-HT receptor antagonist used in the present study was determined by the dose that affects the intestinal motility shown in the previous studies (26, 31), because no previous studies have examined the effects of 5-HT receptor antagonists on intestinal permeability.

Measurement of 5-HT release from vascularly and luminally perfused rat ileum. Male Wistar rats weighing 250–350 g were fasted overnight (16–18 h) before the experiment. Animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (50 mg/kg Nembutal; Abbott Laboratories). The terminal ileum, measuring ~7 cm in length, was prepared for in situ vascular and intraluminal perfusion (9). The blood vessels that supply the stomach, duodenum, jejunum, proximal ileum, colon, pancreas, spleen, kidneys, and adrenal glands were excluded from perfusion. Arterial perfusion was performed by inserting a cannula into the aorta with its tip lying close to the superior mesenteric artery. The aorta was tied off above the celiac artery. The vascular effluent was collected through a portal vein cannula. Luminal perfusion was performed through a cannula inserted into the segment with the tip in the midportion of the segment. This catheter was connected to a transducer (model TP-400T, Nihon Koden), and the pressure was amplified by an ampli- fier (model AP-601G; Nihon Koden). Intraluminal pressure was continuously monitored and recorded on a polygraph (model RM-6100; Nihon Koden).

The vascular perfusate consisted of Krebs solution containing 3% dextan, 0.2% bovine serum albumin, and 5 mM glucose. The perfusate was saturated with 95% O₂-5% CO₂ gas to maintain pH 7.4. The luminal perfusate was 0.1 M PBS, pH 7.4. The flow rate of vascular and luminal perfusion was maintained at 3 and 1 ml/min, respectively. After a 30-min equilibration period, the vascular and luminal effluents were collected for 30 min at 5-min intervals into ice-cold vials. At 18 min, the intraluminal pressure was raised by clamping the luminal effluent. The clamp was released when the pressure reached 40 cmH2O. In some experiments, TTX (Sankyō) was introduced into the vasculature to achieve a final concentration of 1 μM from 12 to 21 min, when the intraluminal pressure was high (≤40 cmH2O).

The level of 5-HT was determined by HPLC. Each 1 ml of effluent was added to 10 μl of 57 mM ascorbic acid, 10 μl of 10 mM disodium EDTA, 10 μl of 1 M perchloric acid, and 10 μl of 51 mM pargyline hydrochloride. Vascular effluent was filtered by using Ultrafree-MC (model 30000 MWL, Nihon Millipore) by centrifuging the samples for 30 min at 10,000 rpm at 4°C. Luminal effluent was filtered manually by using a 0.22-μm-pore disk filter (Milllex-GV; Nihon Millipore). Ali- quotes of 100 μl were subjected to HPLC, and the 5-HT content was measured (12).

Statistical analysis. Statistical analysis of the data shown in Figs. 1, 2, and 3, A and B were performed by using a one-way ANOVA, followed by Fisher’s paired least significant difference test. The degree of changes in the FITC level (%stimulation) was calculated as: (the mean of the FITC-LPS levels in nontreated or saline-injected controls) × 100. A paired t-test (two-tail) was used to compare the values between the saline-injected controls and those induced by chemical infusion. P values of <0.05 were considered significant.

RESULTS

Effect of high intraluminal pressure on FITC-LPS translocation. We injected 50 μg/ml of FITC-LPS into the ileal lumen and determined that the basal FITC-
LPS level in the superior mesenteric vein before the elevation of intraluminal pressure was $9.87 \pm 0.29$ ng/ml ($n = 5$). When we elevated the intraluminal pressure to 40 cmH$_2$O by clamping the luminal effluent, the FITC-LPS level in the superior mesenteric vein significantly increased ($19.09 \pm 0.47$ ng/ml, $n = 5$) to a level $198.4 \pm 10.3\%$ ($n = 5$) of the basal level (Fig. 1).

Effect of high intraluminal pressure on 5-HT release from the vascularly and luminally perfused rat ileum. We examined the effect of elevated intraluminal pressure on 5-HT release in ex vivo vascularly and luminally perfused rat ileum. The basal level of 5-HT release from the perfused ileum into the lumen and the portal circulation was $4.63 \pm 0.37$ ng/ml ($n = 3$) and $3.84 \pm 0.44$ ng/ml ($n = 3$), respectively (Fig. 2). The luminal release of 5-HT significantly increased ($30.86 \pm 3.83$ ng/ml, $n = 3$) with increasing intraluminal pressure (40 cmH$_2$O) to a level 528.0 $\pm$ 39.8$\%$ ($n = 3$) above the basal level. This stimulatory effect of the intraluminal pressure on the luminal release of 5-HT was not altered by the vascular injection of TTX (471.0 $\pm$ 98.1$\%$) (data not shown). The vascular release of 5-HT was not affected by the increasing intraluminal pressure (5.0 $\pm$ 0.6 ng/ml) (Fig. 2).

Effect of 5-HT on FITC-LPS translocation. To clarify the role of 5-HT in the effect of high intraluminal pressure on FITC-LPS translocation, we administered 5-HT into the lumen as well as into the jugular vein. We subsequently measured the FITC-LPS level in the superior mesenteric vein (Fig. 3) and found that the basal level of FITC-LPS in the superior mesenteric vein ($10.48 \pm 0.14$ ng/ml, $n = 6$) significantly increased ($15.76 \pm 0.47$ ng/ml, 152.0 $\pm$ 8.54$\%$ of basal level, $n = 6$) with the intraluminal injection of 10 $\mu$mol$\cdot$kg$^{-1}$•min$^{-1}$ of 5-HT (Fig. 3, A and C). However, the intraluminal administration of 1 and 0.1 $\mu$mol$\cdot$kg$^{-1}$•min$^{-1}$ of 5-HT did not affect the basal level of FITC-LPS in the superior mesenteric vein (Fig. 3C), whereas it significantly increased from $10.48 \pm 0.14$ to $13.08 \pm 0.54$ ng/ml (133.7 $\pm$ 7.25$\%$ of basal level, $n = 6$) after the vascular injection of 0.1 $\mu$mol$\cdot$kg$^{-1}$•min$^{-1}$ of 5-HT (Fig. 3, B and D). The vascular administration of 0.01 $\mu$mol$\cdot$kg$^{-1}$•min$^{-1}$ of 5-HT did not affect the basal level of FITC-LPS detected in the superior mesenteric vein (Fig. 3D).

Effect of 5-HT receptor antagonists on the 5-HT-induced FITC-LPS translocation. To determine which receptor subtypes mediate the effects of 5-HT on FITC-LPS translocation, we intraluminally and intravenously injected 5-HT$_{1/2}$, 5-HT$_{2}$, 5-HT$_{3}$, and 5-HT$_{4}$ receptor antagonists combined with 5-HT, and the FITC-LPS level in the superior mesenteric vein was subsequently measured. The FITC-LPS level in the superior mesenteric vein, which was elevated by intraluminal and intravenous injection of 5-HT, significantly decreased after luminal (Fig. 4, A and D, respectively) or vascular (Fig. 4, B and C, respectively) injection of azasetron, but not by the injection of methysergide, ketanserin or SB-204070.

Effect of 5-HT$_{3}$ receptor antagonists on FITC-LPS translocation induced by high intraluminal pressure. Finally, the involvement of the 5-HT$_{3}$ receptor in the FITC-LPS translocation induced by high intraluminal pressure was examined. We intraluminally and intravenously injected a 5-HT$_{3}$ receptor antagonist (Fig. 5) and simultaneously increased the intraluminal pressure to 40 cmH$_2$O, on which the FITC-LPS level in the superior mesenteric vein was measured. The stimulatory effect of the high intraluminal pressure on FITC-LPS translocation was completely inhibited by both intraluminal and intravenous administration of azasetron (Fig. 5).
DISCUSSION

Our results suggest that the increase in the intraluminal pressure of the rat ileum stimulates the translocation of FITC-LPS from the intestinal lumen to the superior mesenteric vein and that this stimulatory effect is mediated by 5-HT, which is released from EC cells, via 5-HT₃ receptors. We prepared in vivo models of anesthetized rats to measure the rate of translocation, because blood supply and extrinsic nervous innervation in the intestinal segments remain intact in this model (7, 15). For the measurement of 5-HT release from the intestinal segment, we used ex vivo models of
vascularly and luminally perfused ileum, because this preparation is useful to compare the bidirectional release of 5-HT into the lumen and blood vessels (12).

Previous studies (5, 15) have demonstrated that intestinal obstruction causes bacterial translocation in both humans and experimental animals. In these studies, bacterial translocation was evaluated by counting the number of bacteria or radiolabeled E. coli in the mesenteric lymph nodes (5), liver, spleen, or kidney (15). However, endotoxin (LPS) levels in the portal circulation, even in the basal state (30). However, the LPS level detected in the superior mesenteric vein was markedly high compared with the plasma levels of LPS (2–3 pg/ml) in normal animals. This discrepancy may be the result of the induction of mucosal hypertrophy of the portal circulation, even in the basal state (30).

To further identify the epithelial target site of luminal and intravenously administered 5-HT in which vascularly administered 5-HT was more potent than lumenally administered 5-HT. In the ex vivo perfusion study, on the other hand, intraluminal release but not vascular release of 5-HT was stimulated by high intraluminal pressure. This discrepancy can be explained by the fact that 5-HT released from EC cells into the portal circulation was not altered. Because this stimulatory effect was not altered by TTX administration, 5-HT release induced by high intraluminal pressure must originate from EC cells, not from 5-HT-containing enteric neurons (11).

These findings were consistent with previous data that demonstrated that ~0.02% of LPS, applied exogenously into the ileal lumen of rats, was detected in the mesenteric lymph nodes, mesentery, liver, and spleen even in the basal state (30). However, the LPS level detected in the superior mesenteric vein in our study was markedly higher compared with the plasma levels of LPS (2–3 pg/ml) in normal animals. This discrepancy may be the result of the induction of mucosal hypertrophy of the portal circulation, even in the basal state. This method seems reliable, because retranslocation have not been measured. In the present study, we injected FITC-LPS into the ileal lumen, and measured the plasma levels of FITC as an indicator of LPS translocation. Because the mean LPS levels in the luminal content of the terminal ileum of rats under normal conditions is ~1.8 μg/ml, as described previously (30), 50 μg/ml of exogenously applied LPS seems to be a pathological dose. However, this dose is comparable to other in vivo studies, in which 50–400 μg/ml LPS was injected into the intestinal lumen to examine the permeability of endotoxin during noninflammatory stress, such as immobilization, foot shock, or mesenteric ischemia (7, 27, 30). We detected a basal level of 10 ng/ml of FITC-LPS in the superior mesenteric vein, which increased to 200% of the basal level in response to high intraluminal pressure. These results indicate that 0.02% of the total LPS applied into the lumen was translocated into the portal circulation, even in the basal condition.

To further identify the epithelial target site of luminal and intravenously administered 5-HT, 5-HT receptor antagonists combined with 5-HT were injected via the same or different routes. The results showed...
that the effect of luminal injection of 5-HT was inhibited by both luminal and vascular injection of 5-HT_3 receptor antagonist and that the effect of the vascular injection of 5-HT was inhibited by both luminal and vascular injection of 5-HT_3 receptor antagonist. The administration of 5-HT_1, 5-HT_2, and 5-HT_4 receptor antagonists did not affect these results. These results suggest that 5-HT, both intraluminally and intravenously administered, may affect the epithelial cells and increase the permeability via 5-HT_3 receptor, which is possibly localized in the epithelium.

The pathway of endotoxin translocation across the epithelium has been widely investigated in cultured epithelial cells (2, 17), Ussing chamber preparations (25), and Thiry-Vella loops of intestine (29). Proposed pathways include a transcellular route (1, 9) and a paracellular route (17, 25). In the transcellular route, endotoxin is taken by the endocytosis of the epithelial cell and transported to the basolateral side of the epithelium (2). In the paracellular route, the tight junction proteins, such as occludin, ZO-1, and ZO-2, may be involved in the regulation of epithelial permeability (1). 5-HT, both exogenously applied and endogenously released from EC cells, may influence the epithelial cells from an apical and/or basolateral site and stimulate the transcellular route, or may influence the intercellular tight junctions and stimulate the paracellular route, via 5-HT_3 receptors.

Our results demonstrated that FITC-LPS translocation induced by high intraluminal pressure is inhibited by 5-HT_3 receptor antagonist administered both intraluminally and intravenously. However, the localization of the 5-HT_3 receptor on either the apical or basolateral membrane of the epithelial cells has not been demonstrated. A few previous studies demonstrated that 5-HT_3 receptor is localized to the luminal side of the epithelium in the developing intestine (6) and on a small number of endocrine cells in the epithelium (14). Although identification of 5-HT receptors on apical and/or basolateral membrane of the epithelial cells awaits further investigation, 5-HT released from EC cells may function as a critical factor to stimulate the permeability of endotoxin through the intestinal epithelium.

In conclusion, our study demonstrated that the translocation of FITC-LPS injected into the rat ileum and the superior mesenteric vein was stimulated by the increase of intraluminal pressure, and this stimulatory effect might be mediated by 5-HT released from EC cells via the 5-HT_3 receptor possibly located on the epithelium.

REFERENCES


