Slowing intestinal transit by PYY depends on serotonergic and opioid pathways

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PYY, a distal gut peptide released in response to a fatty meal, slows intestinal transit when administered intravenously (47). In addition to this slowing effect on intestinal transit, PYY is considered an inhibitory peptide, because it suppresses gastrin-stimulated acid secretion (1, 42), pancreatic exocrine secretion (1, 40), CCK release (41), and gastric emptying (47). These inhibitory effects of exogenous PYY suggest that it may be responsible for the ileal brake (49), a response that suppresses the digestive activities of the upper gastrointestinal tract when nutrients such as fat reach the distal gut. Immunoneutralization studies (38) confirmed that PYY is a key mediator of the slowing of intestinal transit by the fat-induced ileal brake. It is not known, however, how PYY slows intestinal transit.

Previously, we created a fistulated dog model that divided the small intestine into proximal and distal segments. We used it to show that placing fat in the distal half of small intestine slowed transit in the proximal half of small intestine. Because the luminal content of the proximal and distal intestinal segments were not in continuity, this observation indicated that the fat-induced ileal brake occurred via a reflex, whereby the distal segment exposed to fat served as the afferent limb of the reflex and the proximal segment in which intestinal transit was measured served as the efferent limb. We also showed that delivering ondansetron (a 5-H3 receptor antagonist) (32) or naloxone (a nonspecific opioid receptor antagonist) (59) into the proximal but not distal intestinal segment reversed the fat-induced ileal brake to localize the serotonergic (32) and opioid (59) pathways involved in the fat-induced ileal brake response to the efferent limb of the slowing reflex. In this study, we will test the hypothesis that the slowing of intestinal transit by PYY may depend on similar pathways. Because the slowing of intestinal transit by fat was reversed by ondansetron in conscious dogs (32) and rats (8, 9), we further hypothesized that intestinal transit may be slowed by luminal 5-HT in the whole animal.

The known anatomy of the enteric nervous system would support a close relationship between these pathways. PYY-sensitive Y1 receptors are expressed by neurons of the rat myenteric and submucous plexuses (27). 5-HT synthesizing and releasing neurons are found in the myenteric plexus of guinea pigs (16, 22) with neighboring neurons expressing 5-HT3 receptors (20, 57, 58). In addition, the guinea pig myenteric plexus contains opioid immunoreactive neurons (11, 14, 51, 53) and µ-, κ-, and δ-opioid receptors have all been localized to the myenteric plexus of the dog (2).

Although the relationship between the serotonergic and opioid pathways involved in the slowing of intestinal transit is not known, in vitro experiments showed that opiate-induced contractions in the rat colon continued even after the preparation was made unresponsive to 5-HT (24). This observation would suggest that the response to exogenous opioid is independent of 5-HT. In this study, we tested the hypothesis that intestinal transit may be slowed by an opioid pathway activated by 5-HT neurotransmission.

In this study, using a fistulated dog model, we tested the hypothesis that the slowing of intestinal transit by PYY may depend on serotonergic and opioid pathways.

MATERIALS AND METHODS

General experimental design. With the use of occluding Foley catheters, the small intestine was compartmentalized into the proximal (between fistulas) and distal (beyond midgut fistula) halves of the gut. Buffer (pH 7.0) was perfused into the proximal and distal half of gut, whereas either PYY or saline (control) was delivered intravenously. Ondansetron (a 5-H3 receptor antagonist) or naloxone into either the proximal or distal gut of the proximal gut with marker recovery decreased from 76.2 ± 3.6% (control) to 33.5 ± 2.4% (5-HT) (P < 0.0001) but was reversed by naloxone delivered into the proximal gut with marker recovery increased to 79.9 ± 7.2% (P < 0.0005). We conclude that the slowing of intestinal transit by PYY depends on serotonergic neurotransmission via an opioid pathway.
measured and compared, whereas ondansetron or naloxone was mixed with buffer and delivered into either the proximal or distal half of the gut. The order of testing for each set of experiments followed a separate randomization schedule. For example, in the set of experiments testing for the serotonergic pathway, the four treatments were control, PYY, PYY with ondansetron in the proximal gut (Ond-Prox), and PYY with ondansetron in the distal gut (Ond-Dist). We also tested the relationship between the serotonergic and the opioid pathways by comparing first the effect of luminal 5-HT and second, the effect of luminal naloxone delivered into either the proximal or distal half of the gut on the slowing of intestinal transit by 5-HT.

**Animal preparations.** Procedures used in this study were approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center, Los Angeles, CA. Ten mongrel dogs were each surgically prepared with two chronic intestinal fistulas. Dogs had an average weight of 25 kg. Modified Thomas cannulas were placed into fistulas located ~10 cm distal to the bile and pancreatic ducts (duodenal fistula) and ~160 cm from the pylorus (midgut fistula) (33, 37). With the flanges of the cannula resting against the inner surface of the intestinal wall, the cannulas were fixed against rotation. Just distal to the fistula, a length of Tygon tubing with a diameter of 2 mm was looped around the intestine and fixed by suture through the visceral peritoneum to the intestinal wall. The length of tubing used was varied to be as short as possible without a tightening effect on the lumen. This provided a stent against which an inflated Foley balloon could be pulled to provide a water-tight seal (33). All dogs were given a recovery period of 4 wk and underwent testing only after normal feeding behaviors were reestablished postoperatively. This preparation had good survival, and the dogs remained healthy with stable body weights and unaffected demeanor during their period of participation in this study.

**Experimental preparations.** Dogs were deprived of food but not water for an 18-h period before experiments. Thirty minutes before the start of each experiment, the intestinal cannuas were uncorced so that an occluding Foley catheter could be placed into the distal limb of each of the duodenal and midgut fistulas. By inflating its balloon with ~10 ml of water and cinching the balloon up against the Tygon ring, a water-tight seal was achieved at each fistula (33). The output of each fistula was allowed to drain freely by gravity. Using this method, the proximal (between fistulas) and distal (beyond midgut fistula) half of the gut was compartmentalized.

**Slowing of intestinal transit by PYY.** Phosphate buffer (pH 7.0) was perfused into the proximal and distal half of the gut at 2 ml/min for 90 min. To test for the slowing of intestinal transit, PYY (200 pM·kg⁻¹·h⁻¹) (Bachem, Torrance, CA) or equivolumine (0.15 M NaCl; control) was delivered intravenously for 90 min beginning at the start of intestinal perfusion. This dose of PYY was chosen because it is within the physiological range (100–400 pM·kg⁻¹·h⁻¹) that is effective in increasing intestinal water and electrolyte absorption (4, 5, 39), in delaying phase III of migrating motor complexes (52), in inhibiting gastric acid secretion (19, 56), and in inhibiting pancreatic secretion in dogs (7, 50).

**Testing for the effect of ondansetron and naloxone on PYY-induced slowing of intestinal transit.** To test the hypothesis that the slowing of intestinal transit by PYY depends on a serotonergic pathway, ondansetron (Sigma, St. Louis, MO) was mixed with buffer and delivered into either the proximal or distal half of the gut at the rate of 0.7 mg·kg⁻¹·h⁻¹ (32) over 90 min in five dogs. Naloxone (Sigma) was then used to test the involvement of an opioid pathway in the slowing of intestinal transit by PYY. In separate experiments, instead of ondansetron, naloxone was mixed with buffer and delivered into either the proximal or distal half of the gut at the rate of 0.16 mg·kg⁻¹·h⁻¹ (59) over 90 min in five dogs. The doses of ondansetron and naloxone utilized were selected on the basis of the previously tested dose response of these agents (32, 35). Doses used in this study have been shown to reverse the fat-induced ileal brake (32, 59) and the fat-induced jejunal brake (32, 35). Both ondansetron (32) and naloxone (59) were also previously shown to have no independent accelerating effect on intestinal transit of buffer. Whereas ondansetron is a selective 5-HT₃ receptor antagonist (55), naloxone is a nonspecific opioid receptor antagonist and cannot be used to discriminate among μ-, κ-, and δ-oidiop receptors (15).

**Testing for the effect of luminal 5-HT on intestinal transit.** To test for the slowing of intestinal transit by luminal 5-HT, 0.0033, 0.05, 0.066, or 0.1 mg·kg⁻¹·h⁻¹ 5-HT was delivered over 90 min into the proximal half of the gut in four dogs.

**Testing for the effect of ondansetron on 5-HT-induced slowing of intestinal transit.** To test for the role of 5-HT₃ receptors in the slowing of intestinal transit by luminal 5-HT, 0.1 mg·kg⁻¹·h⁻¹ 5-HT was mixed with buffer and delivered over 90 min into the distal half of gut in four dogs. Concurrently, ondansetron was mixed with buffer and delivered into either the proximal or distal half of gut at the rate of 0.7 mg·kg⁻¹·h⁻¹ over 90 min.

**Testing for 5-HT neurotransmission via opioid pathway.** To test the relationship between the serotonergic and opioid pathways, intestinal transit was measured in five dogs while 0.1 mg·kg⁻¹·h⁻¹ 5-HT was delivered into the distal gut over 90 min. Concurrently, naloxone was mixed with buffer and perfused into either the proximal or distal half of gut at 0.16 mg·kg⁻¹·h⁻¹ over 90 min.

**Measurement of intestinal transit.** To test intestinal transit after the slowing response to PYY is fully activated, 60 min of the 90-min PYY administration period were allowed to pass (38) before the transit marker was administered. Specifically, ~20 μCi technetium-99m-labeled diethylenetriamine pentaacetic acid (99mTc-DTPA) was delivered as a bolus into the proximal half of the gut via the Foley catheter placed in the duodenal fistula to begin measurement of intestinal transit (37, 38). Intestinal transit across the test segment (150-cm length between fistulas) was measured by counting the radioactivity of 1-ml samples collected every 5 min from the output of the midgut fistula for 30 min. Using a matched dose of 99mTc to represent the original delivered bolus, the radioactivity delivered into the segment and the radioactivity of the recovered fistular output were all measured in a gamma well counter (37, 38). After correcting all counts for radioactive decay to time 0, intestinal transit was calculated as the cumulative %recovery of the delivered 99mTc-DTPA over the 30-min collection period.

**Analysis of data.** Each set of experiments was separately analyzed. Intestinal transit was compared as the cumulative %recovery of 99mTc-DTPA over 30 min (means ± SE). The overall effect of treatments was tested with repeated measures one-way ANOVA. To test for the effect of compartmental perfusion of ondansetron or naloxone, intestinal transit was further compared by paired t-tests. The computer program used was Excel (Microsoft).

**RESULTS**

Intestinal transit as represented by the cumulative percent marker recovery of radioactive marker over 30 min is illustrated in Figs. 1–5.

**Effect of ondansetron on PYY-induced slowing of intestinal transit.** Intestinal transit depended on test conditions (P < 0.0005). Intravenous PYY slowed intestinal transit so that marker recovery decreased from 74.1 ± 1.9% (control) to 1.9% (not significant [NS]) (Fig. 1). Slowing of transit by PYY was reversed by ondansetron in the proximal half of the gut with marker recovery increased from 11.4 ± 3.8% PYY (P < 0.0001). In contrast, Ond-Dist did not have any noticeable effect on the PYY-induced slowing of transit as shown by the marker recovery of 20.6 ± 4.9% [not significant (NS)] (Fig. 1).

**Effect of naloxone on PYY-induced slowing of intestinal transit.** Intestinal transit depended on test conditions (P < 0.001). PYY delivered intravenously slowed intestinal transit...
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Fig. 1. Peptide YY (PYY)-induced slowing of intestinal transit was reversed by luminal ondansetron. PYY or saline (control) was delivered intravenously at the start of 90-min intestinal perfusion. Intestinal transit was represented by cumulative recovery of marker during the last 30 min of the 90-min experiment. Ondansetron was mixed with buffer and delivered into either the proximal (Ond-Prox) or distal (Ond-Dist) half of the gut during PYY administration. Compared with control, PYY slowed intestinal transit to decrease cumulative recovery of marker. Ondansetron delivered into the proximal (Ond-Prox) but not distal (Ond-Dist) half of the gut reversed PYY-induced slowing of intestinal transit. Data are the mean values of 5 dogs.

Effect of ondansetron on 5-HT-induced slowing of intestinal transit. Buffer alone was perfused through the proximal and distal half of the gut as the control. Compared with control, 5-HT delivered into the distal half of the gut slowed intestinal transit by 5-HT. Data are the mean values of 4 dogs.

Fig. 2. PYY-induced slowing of intestinal transit was reversed by luminal naloxone. Naloxone was mixed with buffer and delivered into either the proximal (Nal-Prox) or distal (Nal-Dist) half of the gut during PYY administration. Compared with control, PYY slowed intestinal transit to decrease cumulative recovery of marker. Nal-Prox but not Nal-Dist reversed PYY-induced slowing of intestinal transit. Data are the mean values of 5 dogs.

Fig. 3. Intestinal transit is slowed by 5-HT mixed with buffer and delivered into the proximal half of the gut at the rate of 0 (control), 0.033, 0.05, 0.066, and 0.1 mg·kg⁻¹·h⁻¹ as represented by decreasing cumulative recovery of marker. Data are the mean values of 4 dogs.

Fig. 4. Ondansetron reversed slowing of intestinal transit by 5-HT. Buffer alone was perfused through the proximal and distal half of the gut as the control. Compared with control, 5-HT delivered into the distal half of the gut slowed intestinal transit. Ondansetron was mixed with buffer and delivered into either the proximal or distal half of the gut. Ond-Prox reversed 5-HT-induced slowing of intestinal transit. Ond-Dist also reversed slowing of intestinal transit by 5-HT. Data are the mean values of 4 dogs.

DISCUSSION

In a fistulated dog model, we found that the slowing of intestinal transit by PYY was reversed by ondansetron (a 5-HT₃ receptor antagonist) and by naloxone (a nonspecific opioid receptor antagonist) when either of these agents were...
delivered luminally into the small intestine. However, these antagonists reversed the slowing of transit by PYY only when they were delivered into the proximal half of the small intestine in which transit was measured. This compartment-specific effect suggests that the serotonergic and opioid pathways involved in the slowing of intestinal transit by PYY are located on the efferent limb of the slowing reflex response.

Previously, we reported in the same model that slowing of intestinal transit by fat in the distal gut as the fat-induced ileal brake depended on the release of PYY (38). In addition, we found that a serotonergic pathway (32) and an opioid pathway (59) located on the efferent limb of this slowing response were required. We were able to demonstrate this localization by compartmentalizing the proximal from the distal half of the small intestine (32, 59). This approach separated the distal segment of the intestine that was perfused with fat from the proximal segment that was used to measure intestinal transit. Because intestinal transit was only measured in the proximal compartment, the proximal segment served as the efferent limb of the slowing reflex response. Correspondingly, the distal segment served as the afferent limb of the reflex response. Similarly, in this study, we found that the same dose of ondansetron or naloxone reversed the fat-induced ileal brake only when these antagonists were perfused into the proximal but not the distal segment of the small intestine to localize the pathway to the efferent limb. We were also able to conclude that these pathways must be located in the enteric nervous system rather than the central nervous system (32, 59).

The compartment-specific effect is the strongest evidence that ondansetron or naloxone worked locally and not systemically, because identical amounts of each antagonist were delivered into each segment, but these agents only reversed the ileal brake when delivered into the efferent but not afferent limb of the slowing reflex. This finding simply cannot be explained by a postabsorptive systemic effect.

In this study, we were able to observe a similar compartment-specific effect with luminally administered ondansetron or naloxone even as PYY was administered intravenously to slow intestinal transit. Specifically, the slowing response to PYY was reversed when either antagonist was delivered into the compartment used for the measurement of intestinal transit (proximal but not the distal half of the gut). Although afferent and efferent nerves were both present in each compartment, we were again able to localize these pathways to the efferent limb, because intestinal transit was measured only in the proximal compartment. Thus a disruption of a pathway on the efferent limb of the reflex can only be observed if the antagonist were to be delivered into the compartment in which transit was measured. The inhibitory action of PYY on intestinal transit is then similar to the effect of this peptide on pancreatic secretion, which is also known to be dependent on a neural pathway located on the efferent limb (43).

This accelerating effect of ondansetron on small intestinal transit may be specific for the disruption of the fat-induced or PYY-induced slowing response, because this accelerating effect was not seen in the absence of nutrients in the dog (32). In addition, ondansetron has been reported to slow transit in the fasting state in mice (45, 46) and rats (12).

There are three enteric sites of response to 5-HT: 1) the intrinsic primary afferent neuron (IPAN) responsible for the peristaltic reflex and secretion (3, 13, 25, 28, 44); 2) extrinsic primary sensory nerves responsible for gut-to-central nervous system (6) or gut-to-pancreas (29, 30, 31) communications; and 3) myenteric neurons that express 5-HT3 receptors (20, 22, 44, 57). The first two sites (IPAN and extrinsic sensory nerve) are obviously located on the efferent limb of the response, but the myenteric neurons that participate in serotonergic neurotransmissions are located on the efferent limb of the response (32). These myenteric neurons operate on the efferent limb, because they are interneurons that receive extrinsic innervation from noradrenergic fibers (23) and provide input to neighboring neurons.

These myenteric neurons express 5-HT3 receptors (20, 22, 44, 57) and receive neurotransmission from 5-HT synthesizing and releasing neurons first described by Gershon et al. (22). Previously, the physiological function of 5-HT neurotransmission via these myenteric neurons was not known (21). By localizing the ondansetron-sensitive serotonergic pathway involved in the fat-induced ileal brake to the efferent limb, we were able to demonstrate the involvement of myenteric neurons in 5-HT neurotransmission (32). The physiological function of this third site of response to 5-HT is therefore the slowing of small intestinal transit (32).

This study extends our understanding of the role of these efferent, serotonergic pathways. 5-HT neurotransmission via myenteric neurons is then involved in the slowing of small intestinal transit by fat or PYY. The idea that a serotonergic pathway is involved in the slowing of intestinal transit is not well known. The possibility that 5-HT may slow small intestinal transit in whole animals was suggested by the acceleration of transit by ondansetron in both dogs (32) and rats during fat-induced ileal brake (8, 9). In this study, we used luminal perfusion of 5-HT to directly test the hypothesis that luminal 5-HT may slow transit in the whole animal. We found that luminal 5-HT did indeed slow transit in a dose-dependent fashion. This slowing effect of 5-HT in whole animals contrasts with the triggering of peristalsis (accelerates transit) by 5-HT in in vitro models (10, 25, 48). A comparison of the different effects of 5-HT in the in vitro models vs. that in whole animals would suggest that the slowing of intestinal transit by luminal 5-HT may depend on extrinsic nerves that participate...
in long, intestinointestinal reflexes. These nerves would be present in the whole animals but absent in vitro models.

We found that the slowing of intestinal transit by distal gut 5-HT was reversed by Ond-Prox or Ond-Dist. In contrast to the slowing response to fat or PYY, the slowing response to distal gut 5-HT involves 5-HT₃ receptors located in both the afferent and efferent limbs of the reflex response.

Ondansetron delivered with 5-HT into the distal gut had a detectable effect in abolishing the slowing effect of 5-HT as early as 10 min into the measurement (Fig. 4). However, this immediate effect is in contrast to ondansetron delivered into the distal gut in which the accelerating effect of the 5-HT₃ receptor antagonist was not evident until 30 min. A possible explanation for this difference may be related to the availability of 5-HT in the distal vs. proximal compartment. In the distal compartment, 5-HT was immediately available to respond to the antagonist, because this amine was delivered directly. In the proximal compartment, 5-HT became available only after 5-HT was released from the proximal gut in response to 5-HT in the distal gut (34).

Opioid immunoreactivities have been observed in both myenteric and submucosal plexuses of the small intestine in dogs (2). Opioid pathways have been reported to inhibit the excitatory motoneurons (17, 54) as well as the inhibitory enteric and submucosal plexuses of the small intestine in dogs in the distal gut (34).

5-HT was released from the proximal gut in response to 5-HT because this amine was delivered directly. In the distal compartment, 5-HT involved 5-HT₃ receptors located in both the afferent and efferent limbs of the reflex response. Ondansetron delivered with 5-HT into the distal gut had a detectable effect in abolishing the slowing effect of 5-HT as early as 10 min into the measurement (Fig. 4). However, this immediate effect is in contrast to ondansetron delivered into the distal gut in which the accelerating effect of the 5-HT₃ receptor antagonist was not evident until 30 min. A possible explanation for this difference may be related to the availability of 5-HT in the distal vs. proximal compartment. In the distal compartment, 5-HT was immediately available to respond to the antagonist, because this amine was delivered directly. In the proximal compartment, 5-HT became available only after 5-HT was released from the proximal gut in response to 5-HT in the distal gut (34).

Opioid immunoreactivities have been observed in both myenteric and submucosal plexuses of the small intestine in dogs (2). Opioid pathways have been reported to inhibit the excitatory motoneurons (17, 54) as well as the inhibitory motoneurons (18, 26) involved in the peristaltic reflex. These observations suggest that intestinal transit may be slowed through modifying the peristaltic reflex via the activation of opioid nerves. Because opiate-induced contractions in the rat colon continued even after the preparation was made unresponsive to 5-HT (24), the action of opiate was independent of 5-HT. In this study, we hypothesized that the opioid pathway may be located distal to the 5-HT pathway in the intestine, i.e., the serotonergic pathway may slow intestinal transit by acting on an opioid pathway. In this study, we confirmed this arrangement by showing that the slowing of intestinal transit by 5-HT was reversed by naloxone. Similar to its effect on the ileal brake, we found that naloxone reversed the slowing response to distal gut 5-HT only when the antagonist was available to the compartment in which intestinal transit was measured (localizing the opioid pathway to the effenter limb). We conclude that the slowing of intestinal transit by PYY depends on serotonicergic and opioid pathways.

In summary, we extended our previous reports that the fat-induced ileal brake depended on PYY (38) as well as a serotonergic (32) and an opioid (39) pathway by showing that PYY activates serotonergic and opioid pathways. By linking these findings with our recent observation that the slowing of intestinal transit by fat or PYY depends on a β-adrenergic pathway (36), we are able to conclude that the fat-induced ileal brake is a sequence of events involving gut peptides and nerves. Specifically, the reflex response begins with luminal fat and ends with the slowing of intestinal transit with the slowing signal carried successively by PYY, β-adrenergic pathway, a serotonergic pathway, and an opioid pathway.

REFERENCES


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