Slowing of intestinal transit by fat or peptide YY depends on β-adrenergic pathway

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INTESTINAL TRANSIT IS SLOWED by fat in the proximal and distal half of gut. The role of adrenergic, serotoninergic, and opioid pathways was then tested in the slowing of intestinal transit by fat, PYY, and norepinephrine. Intestinal transit results were compared as the cumulative percent marker of recovery over 30 min. We found that the slowing of transit by fat, PYY, or norepinephrine was reversed by propranolol. In addition, the slowing effect of fat was reversed by metoprolol (β1-adrenoreceptor antagonist) but not phentolamine (α-adrenoreceptor antagonist). Furthermore, norepinephrine-induced slowing of transit was reversed by ondansetron (5-HT3 receptor antagonist) or naloxone (opioid receptor antagonist). Extending these physiological results, we also found by immunohistochemistry that β1-adrenoreceptors are expressed by neurons of the intrinsic plexuses of the small intestine. We conclude that the slowing of intestinal transit by fat or PYY depends on a β-adrenergic pathway and that this adrenergic pathway acts on serotoninergic and opioid pathways.
ways. The order of testing followed a randomization schedule. Once the physiological results were available to pinpoint the specific adrenoreceptors involved in the slowing effect, immunohistochemical techniques were used to test for the expression of such adrenoreceptors in the small intestine.

**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. Eighteen mongrel dogs were each surgically prepared with two chronic intestinal fistulas. Dogs averaged 25 kg in body weight. Modified Thomas cannulas were placed into fistulas located ~10 cm (duodenal fistula, distal to the bile and pancreatic ducts) and ~160 cm (midgut fistula) from the pylorus (16, 18). 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 individualized to be as short as possible without a tightening effect on the lumen. This provided a stent against which an infusion could be pulled and maintained without tight seal. To perform immunohistochemistry, a full thickness biopsy of the small intestine was performed during the initial surgery in five dogs. The specimen was immediately immersed in 4% paraformaldehyde for tissue fixation. 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 for >12 mo of observation.

**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 cannulas were uncurked so that a 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 with the Foley catheter (16). The output of each fistula was allowed to drain freely by gravity. With the use of this method, the proximal (between fistulas) and distal (beyond midgut fistula) half of gut was compartmentalized.

**Intestinal perfusates.** To trigger the fat-induced ileal brake, 60 mM oleate as a 300 mosM solution of mixed micelles with monolein and 10 mM taurocholate in pH 7.0 phosphate buffer at room temperature was delivered into the distal half of gut via the Foley catheter in the duodenal fistula at 2 ml/min for 90 min, with buffer (pH 7.0) alone delivered into the proximal half of gut via the catheter in the duodenal fistula at 2 ml/min for 90 min (18, 19). In experiments in which intravenous PYY, NE, or saline was administered, pH 7.0 buffer alone was perfused into the proximal and distal half of gut at 2 ml/min.

**Intravenous test agents.** Doses of the test agents were selected on the basis of their effectiveness in dogs. In experiments testing the transit response to PYY or NE, either PYY or NE was delivered intravenously at 200 μM·kg⁻¹·h⁻¹ (2, 3, 22) for 90 min, or NE was administered intravenously at 0.6 mg·kg⁻¹·h⁻¹ (36) over 90 min. The control was 0.15 M NaCl.

**Testing for the involvement of a β₁-adrenoreceptor pathway.** Three separate studies were completed: 1) the effect of propranolol on fat-induced ileal brake (n = 5), 2) the effect of propranolol on PYY-induced slowing of intestinal transit (n = 4), and 3) the effect of propranolol on NE-induced slowing of intestinal transit (n = 5). Propranolol was delivered intravenously at 50 μg·kg⁻¹·h⁻¹ for 90 min (28). The control was 0.15 M NaCl.

**Testing for the role of serotonergic and opioid pathways.** The slowing of intestinal transit by fat depends on an ondansetron-sensitive serotonergic (21) and a naloxone-sensitive opioid pathway (38) located on the efferent limb of the reflex response. To test the hypothesis that the slowing of intestinal transit by NE depends on a similar serotonergic pathway, intestinal transit during intravenous NE administration at 0.6 mg·kg⁻¹·h⁻¹ (36) for 90 min was compared with saline or ondansetron, the 5-HT3 receptor antagonist, mixed with buffer and delivered into the proximal half of gut at 0.7 mg·kg⁻¹·h⁻¹ (21) for 90 min (n = 5). To test the role of an opioid pathway, saline or naloxone the opioid receptor antagonist was mixed with buffer and delivered at a dose of 0.16 mg·kg⁻¹·h⁻¹ for 90 min (n = 5) (38). The control was 0.15 M NaCl.

**Testing for the role of β₁ vs. α-adrenoreceptor pathway.** In separate experiments, to compare the involvement of a β₁-vs. α-adrenoreceptor pathway in the slowing of transit by the ileal brake, we compared the effect of metoprolol (specific β1-adrenoreceptor antagonist) delivered at 50 μg·kg⁻¹·h⁻¹ (1) over 90 min and phentolamine (nonspecific α-adrenoreceptor antagonist) delivered at 50 μg·kg⁻¹·h⁻¹ (28) over 90 min during the ileal brake. NaCl (0.15 M) was administered intravenously as the control.

**Measurement of intestinal transit.** Sixty minutes after the start of the 90-min perfusion (to allow time for full activation of inhibitory feedback) (18, 19), ~20 μCi 99m-Tc-DTPA was delivered as a bolus into the test segment to begin measurement of intestinal transit (18, 19). Intestinal transit across the proximal half of gut (150 cm length) was measured by counting the radioactivity of 1-ml samples collected every 5 min from the output of the midgut fistula for 30 min. With the use of a matched dose of 99m-Tc-DTPA to represent the original delivered bolus, the radioactivity delivered into the segment and the radioactivity of the recovered fistulous output were both measured in a gamma well counter (18, 19). After correcting all counts to time 0, intestinal transit was calculated as the cumulative %recovery of the delivered 99m-Tc-DTPA over the 30-min collection period.

**Testing for β₁-adrenoreceptor immunoreactivity in the dog intestine.** Once the physiological results demonstrating the role of a β₁-adrenoregic pathway were available, we used immunohistochemistry to test for the expected anatomy. Specifically, tissues were obtained from the jejunum of five dogs for immunohistochemical studies to identify β₁-adrenoreceptor immunoreactivity. Tissues were fixed with 4% paraformaldehyde in 0.1 M phosphate buffer and then incubated in phosphate buffer with 15% sucrose at 4°C for 15 h. The tissue was embedded in paraffin and cut into transverse sections. Tissue was processed for immunohistochemistry using standard immunohistochemical methods (34). The primary antibody was an affinity-purified polyclonal rabbit antisemur raised against human β₁-adrenoreceptors (A-20 (model sc-567; Santa Cruz Biotechnology, Santa Cruz, CA). The standard ABC method (34) was employed. The stain was completed after incubation with Retrieve-All (Signet Laboratories) followed by 3% H₂O₂. The specificity of this antibody against β₁-adrenoreceptor has been confirmed by immunohistochemistry and Western blotting (Data Sheet; Santa Cruz Biotechnology). Images were captured with the use of a standard light microscope (Olympus Bx 60) at the final ×200 magnification and digitized and stored on hard disk by using MagnaFire SP software (Optronics, Goleta, CA). β₁-adrenoreceptor staining was visualized as brown color in images of tissue counterstained with hematoxylin to
show the background immunoreactivity. Control specimens were processed identically except that the tissue was not incubated with the primary antibody to the β1-adrenoceptor.

**Analysis of data.** Intestinal transit results were compared as the cumulative %recovery of 99m-Tc-DTPA over 30 min. To test for the effect of the test condition, a one-way ANOVA was used. To test for the effect of an antagonist vs. control, a paired Student’s t-test was used. The computer program used was Excel (Microsoft). *P* values were considered to be significant if *p* < 0.05.

**RESULTS**

Intestinal transit was represented as the cumulative percent recovery of a radioactive marker over 30 min (%recovered) (Figs. 1–6).

The effect of propranolol on the fat-induced ileal brake. Intestinal transit depended on the test conditions (*P* < 0.001) (Fig. 1). Intestinal transit was slowed by perfusing oleate into the distal half of gut as the fat-induced ileal brake. Marker recovery decreased from 70.1 ± 6.5% with buffer (control) to 26.6 ± 5.4% with oleate in the distal half of gut (oleate) (*P* < 0.005). The ileal brake was reversed by propranolol with marker recovery increased from 26.6 ± 5.4 (oleate) to 66.4 ± 8.3% (propranolol) (*P* < 0.05).

Effect of propranolol on PYY-induced slowing of intestinal transit. Intestinal transit depended on the test conditions (*P* < 0.00001) (Fig. 2). Intestinal transit of buffer through gut was slowed by intravenous PYY. Marker recovery decreased from 75.0 ± 4.4 (control) to 15.3 ± 6.3% (PYY) at 30 min (*P* < 0.001). Slowing of intestinal transit by intravenous PYY was reversed by intravenous propranolol with marker recovery increased from 15.3 ± 6.3 (PYY) to 72.2 ± 4.8% (PYY - propranolol) (*P* < 0.01).

Effect of propranolol on NE-induced slowing of transit. Intestinal transit depended on test conditions (*P* < 0.00001) (Fig. 3). Intestinal transit of buffer was slowed by intravenous NE. Marker recovery decreased from 71.4 ± 1.8 (control) to 26.9 ± 1.8% (NE) after intravenous administration of NE (*P* < 0.01). NE-induced slowing of transit was reversed by intravenous propranolol with marker recovery increased from 26.9 ± 1.8 (NE) to 65.9 ± 3.1% (NE - propranolol) (*P* < 0.005).

Testing for the role of serotonergic pathway. Intestinal transit depended on test conditions (*P* < 0.00001) (Fig. 4). Intestinal transit was slowed by intravenous NE with marker recovery decreased from 68.5 ± 5.0 (control) to 16.3 ± 3.4% (NE) (*P* < 0.001). The NE-induced slowing of transit was reversed by luminal ondansetron with marker recovery increased from 16.3 ± 3.4 (NE) to 63.0 ± 4.4% (NE - ondansetron) (*P* < 0.001).

Testing for the role of opioid pathway. Intestinal transit depended on test conditions (*P* < 0.00001) (Fig. 5). Intestinal transit was slowed by intravenous NE with marker recovery decreased from 74.6 ± 3.1 (control) to 20.2 ± 3.5% (NE) (*P* < 0.001). The NE-induced slowing of transit was reversed by luminal naloxone so...
that the marker recovery increased from 20.2 ± 3.5 (NE) to 63.7 ± 6.2% (NE - naloxone) (P < 0.005).

Testing for the role of β₁- vs. α-adrenergic pathway. Intestinal transit depended on test conditions (P < 0.00001) (Fig. 6). Intestinal transit was slowed by olate in the distal half of gut as the ileal brake with marker recovery decreased from 68.3 ± 2.6 (control) to 27.5 ± 2.4% (oleate) (P < 0.005). We found that metoprolol reversed slowing of intestinal transit by fat with marker recovery increased from 27.5 ± 2.4 (oleate) to 62.1 ± 3.7% (metoprolol) (P < 0.01). The ileal brake with marker recovery of 27.5 ± 2.4% (oleate) was not affected by intravenous phentolamine with marker recovery of 31.8 ± 4.1% (phenololamine) (not significant).

Testing for β₁-adrenoreceptor immunoreactivity in the dog intestine. β₁-adrenoreceptor immunoreactivity was observed on smooth muscle cells of the longitudinal and circular muscle of the muscularis externa (Fig. 7A) and the muscularis mucosae (Fig. 7C). β₁-adreno-

DISCUSSION

In the present study, we identified a novel metoprolol-sensitive β₁-adrenergic pathway involved in the slowing of intestinal transit by fat. Although the role of an α₂-adrenergic pathway has been well studied in the response of intestinal motility to mechanical stimuli (15, 27, 31), this is the first report of the involvement of a β₁-adrenergic pathway in the intestinal transit response to the chemical stimuli of fat. Our conclusion is supported by physiological data showing that the slowing of intestinal transit by fat or PYY was abolished by propranolol, the nonspecific β-adrenergic receptor antagonist. In addition, we found that the fat-induced ileal brake was reversed by metoprolol, the β₁-adrenergic receptor antagonist but not phenolamine, the nonspecific α-adrenergic receptor antagonist, thus demonstrating the role for a metoprolol-sensitive β₁-adrenergic pathway in this slowing response. Our conclusion is also supported by anatomical data showing that β₁-adrenoreceptor immunoreactivity is found on neurons of the intrinsic plexuses of the small intestine.

Previously, we reported that the slowing of intestinal transit by fat was dependent on PYY on the afferent limb of the slowing reflex response (19) and a serotonergic (21) as well as an opioid pathway (38) on the efferent limb of this reflex. These studies provided evidence for the grouping together of PYY, a serotonin-
Fig. 7. A: $\beta_1$-adrenoreceptor immunoreactivity (brown color) is localized to neurons (arrow points to one neuron) of the myenteric plexus and to smooth muscle cells of the longitudinal and circular muscle layers of the muscularis externa. Below and to the right of the arrow are two stained cells likely to be enteric neurons; however, the nuclei are not visible due to the plane of section. Images were captured at ×200 magnification. B: enlarged view of the neuron demonstrating $\beta_1$-adrenoreceptor immunoreactivity (arrow) within the myenteric plexus. The yellow, boxed area from Fig. 7 is shown as an enlarged image here. C: $\beta_2$-adrenoreceptor immunoreactivity (brown in color) is found on neurons of the submucous plexus (arrow), smooth muscle cells of the muscularis mucosae, and the wall of blood vessels. There is a lack of staining of the enterocytes of the mucosa and the lamina propria. Images were captured at ×200 magnification. D: lack of brown color staining in control tissue specimen which was processed by repeating all the steps of immunohistochemistry except incubation with primary antibody to the $\beta_1$-adrenergic receptor. There is no staining of circular or smooth muscle cells or the longitudinal muscles. No staining was observed on any neurons of the myenteric plexus. Images were captured at ×200 magnification.

ergic pathway and an opioid pathway in the slowing of intestinal transit by fat.

We did not know, however, how the slowing signal (fat or PYY) was carried from the afferent limb to the efferent limb of this reflex response. A noradrenergic nerve acting on $\alpha_2$-adrenoreceptors is known to link the afferent and efferent limbs of the inhibitory reflex triggered by mechanical stimulation (27, 31). This enteric inhibitory reflex depends on both intrinsic and extrinsic nerves. Specifically, the inhibitory signal generated by the mechanical stimulation of one segment of the intestine is first carried from the gut to a prevertebral ganglion (15) via a cholinergic nerve. The signal is then carried back to the gut from the prevertebral ganglion via a postganglionic adrenergic nerve (31) that acts to inhibit another segment of the intestine. This mechanosensitive, adrenergic pathway acts on $\alpha_2$-adrenoreceptors (27) to inhibit intestinal motility (9, 10, 31) and secretion (4, 35).

Although it is reported that adrenergic nerves are not activated by the usual meal conditions (9), there is evidence for a postprandial role for these noradrenergic nerves. There are indeed observations that cannot be explained if inhibition of motility were to be the only response to adrenergic input. Specifically, in humans, by using the $\beta$-agonist isoprenaline, intestinal motility was switched from the fasting to the fed pattern (33) to suggest that a $\beta$- rather than an $\alpha$-adrenergic pathway may be active in the fed motility state. The existence of a separate, stimulatory $\beta$-adrenergic pathway was further suggested by the observation that the contractile response of the small intestine to acetylcholine was enhanced by a $\beta$-adrenoreceptor agonist (24).

Previously, we reported that the slowing of intestinal transit by fat depended on an ondansetron-sensitive serotonergic pathway (21) as well as a naloxone-sensitive opioid pathway (38) located on the efferent limb of the slowing reflex. Specifically, the efferent location and the response to ondansetron suggested the involvement of serotonergic neurotransmission via myenteric neurons that express 5-HT3 receptors (21) including the serotonin synthesizing and releasing neurons of the myenteric plexus described by Gershon et al. in 1965 (12). Serotonergic neurons represent a subpopulation of cells in the myenteric plexus (25). We now extend these observations by showing that the slowing of intestinal transit by NE, the adrenergic neurotransmitter, is similarly reversed by luminal ondansetron or naloxone. These observations along with our finding that the slowing of intestinal transit by fat is reversed by metoprolol but not by phentolamine provided the evidence for the grouping of PYY, a noradrenergic nerve acting on $\beta_1$-adrenoreceptors, a serotonergic pathway, and an opioid pathway in the slowing of intestinal transit by fat.

In this study, using an immunohistochemical approach, we found $\beta_1$-adrenoreceptor immunoreactivity to be present on the cell bodies of neurons of the myenteric plexus and the submucous plexus. $\beta_1$-adrenoreceptor immunoreactivity was also localized to the smooth muscles of the muscularis mucosae, circular muscles, and longitudinal muscles of the intestine (37).
as well as the vasculature (8) as would be expected from the known adrenergic innervation of these structures. Although the expression of $\beta_1$-adrenoreceptors by neurons of the intrinsic plexuses of the intestine has not been previously reported, noradrenergic nerves are known to terminate onto neurons of the myenteric and submucous plexuses (11, 13, 23). Neuroanatomy of adrenergic innervation of the small intestine also supports a relationship between a noradrenergic nerve and a serotonergic neuron because noradrenergic nerves are reported to terminate onto 5-HT synthesizing and releasing neurons of the myenteric plexus (11) as well as on cholinergic motor neurons and muscles (27). Although the physiological function of this noradrenergic innervation of serotonergic neurons was not previously known. Our results suggest that the slowing of intestinal transit by fat is the physiological function of noradrenergic neurotransmission via serotonergic neurons.

Previously, we reported that the response to luminal 5-HT in in vitro models differs from that seen in whole animals (21). Although intestinal peristalsis leading to acceleration of transit is the observed response to serotonin in in vitro models (7, 14, 29), the observed effect of serotonin in conscious animals is the slowing of intestinal transit (5, 6). Because extrinsic nerves are severed in in vitro models but are available in the whole animal, the different responses to serotonin in these models can be explained whether extrinsic nerves are involved in the slowing of intestinal transit by fat. The findings from this study would suggest that the required extrinsic nerve in the slowing of transit by fat is the noradrenergic nerve that acts on $\beta_1$-adrenoceptors.

DISCLOSURES

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