**Slowing of intestinal transit by fat depends on naloxone-blockable efferent, opioid pathway**

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ZHAO, XIAO-TUAN, LJIE WANG, AND HENRY C. LIN. Slowing of intestinal transit by fat depends on naloxone-blockable efferent, opioid pathway. Am J Physiol Gastrointest Liver Physiol 278: G866–G870, 2000.—Slowing of transit through the proximal small intestine by fat in the distal gut is termed the ileal brake. Intravenous naloxone, an opioid receptor antagonist, abolished the fat-induced ileal brake, suggesting that an endogenous opioid pathway may be involved in this response. To test the hypothesis that slowing of intestinal transit by fat in the distal half of the gut depends on an opioid pathway located on the effenter limb of this response, we compared intestinal transit in dogs equipped with duodenal and midgut fistulas while naloxone was either compartmentalized with oleate to the distal half of the gut or with buffer to the proximal half of the gut. We found that intestinal transit depended on the perfusion conditions (P < 0.00001). Specifically, compared with ileal brake (marker recovery of 35.7 ± 7.4%), intestinal transit was accelerated when naloxone was delivered into the proximal half of the gut (76.2 ± 5.2%) (P < 0.005) but not the distal half of the gut (29.4 ± 5.4%). We conclude that slowing of intestinal transit by fat in the distal half of the gut depends on an opioid pathway located on the effenter limb of the ileal brake.

THE SLOWING OF TRANSIT THROUGH THE PROXIMAL SMALL INTESTINE DURING FAT PERFUSION OF THE DISTAL SMALL INTESTINE IS TERMED THE ILEAL BRAKE (9, 27, 29). Intravenous infusion of naloxone, an opioid receptor antagonist, abolished the slowing effect of ileal fat, suggesting that an opioid pathway may be involved in the fat-induced ileal brake (2, 19). Because intravenous naloxone may have accelerated gastrointestinal transit by acting on opioid receptors located either peripherally (gut) (10, 31) or centrally (brain) (25), the location of this naloxone-blockable opioid pathway for the fat-induced ileal brake is unknown. Opioid receptors are found on ganglion cells of myenteric and submucosal plexuses of the small intestine as well as on intramural nerve fibers in dogs and humans (1, 28). Accordingly, a peripherally located opioid pathway may be important in the response of the ileal brake to naloxone. To trigger the fat-induced ileal brake, fat must be sensed by the distal small intestine (afferent limb) and intestinal transit must be slowed by the proximal small intestine (effenter limb). Orally administered naloxone has little systemic bioavailability (5, 20), so through compartmentalizing intestinal exposure to this antagonist, the role of the opioid pathway in the effenter vs. effenter limb of this response may be tested. In this study in dogs equipped with duodenal and midgut fistulas, we tested the hypothesis that the slowing effect of intestinal transit by fat in the distal half of the gut depends on an opioid pathway located on the effenter limb of this response. We compared intestinal transit while naloxone was delivered into either the proximal (effenter limb) or distal (affenter limb) half of the gut.

METHODS

General experimental design. Dogs were equipped with duodenal and midgut fistulas. In 11 dogs, 60 mM oleate was perfused into the distal half of the gut (beyond midgut fistula) while phosphate buffer (pH 7.0) was perfused into the proximal half of the gut (between fistulas). Intestinal transit across the proximal half of the gut was compared while 6 mg of naloxone were delivered with buffer into the proximal half of the gut (effenter limb) or with oleate into the distal half of the gut (affenter limb). No naloxone was administered into either half of the gut as the positive control experiment (ileal brake). Four of the eleven dogs were tested with buffer perfused into both the proximal and distal halves of the gut as the negative control experiment (buffer control) and also with naloxone added to the buffer as the validation experiment to exclude a nonspecific accelerating effect of the antagonist. The order of testing followed a randomization schedule.

Animal preparations. The procedures used in this study were approved by the Institutional Animal Care and Use Committee at Cedars-Sinai Medical Center (Los Angeles, CA). Eleven mongrel dogs were each surgically prepared with two chronic intestinal fistulas. Dogs weighed an average of 25 kg. 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 (21). 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 (2 mm diameter) 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 inflated Foley balloon could be pulled to provide a water-tight seal. All dogs were given a recovery period of 4 wk and underwent testing only after normal feeding behaviors were reestab-
lished postoperatively. This preparation had good survival, and the 11 dogs remained healthy with stable body weights and unaffected demeanor for more than 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 uncoiled so that a Foley catheter could be placed into the distal limb 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 (21). The output of each fistula was allowed to drain freely by gravity. Using this method, the proximal (between fistulas) and distal (beyond midgut fistula) halves of the gut were compartmentalized. All experiments were carried out in the fed motility state by feeding dogs a can of dog food at the start of the perfusion and then diverting the gastric output completely at the duodenal fistula so that intestinal transit was determined by the intestinal perfusate alone (22, 24). This was done to ensure that transit was always tested in the fed motility state even during nonnutrient-containing containing buffer control.

To trigger fat-induced ileal brake, 60 mM oleate as a 300 mosmol/kgH2O solution of mixed micelles with monolein and 10 mM taurocholate in phosphate buffer (pH 7.0) at room temperature was delivered into the distal half of the gut via the catheter in the midgut fistula (21) and buffer alone was delivered into the proximal half of the gut via the catheter in the duodenal fistula. The caloric content of the perfused oleic acid was 15.2 kcal/100 ml. Although the use of the term ileal brake for the delivery of fat to the distal half of the gut is consistent with the published literature (22, 27, 29), the feedback response from the distal half of the gut as described in this study is not from the ileum alone.

To test for the role of an opioid pathway located in the afferent vs. efferent limb, 6 mg of naloxone hydrochloride (Sigma Chemical, St. Louis, MO) were dissolved in either the oleate or buffer solution and perfused according to the randomization schedule. With the occluding Foley catheters in place, intestinal exposure to naloxone was then compartmentalized to either the proximal (buffer) or distal (oleate) half of the gut. The perfusates were delivered at 2 ml/min for 90 min. In the positive control experiment (ileal brake), no naloxone was administered with either buffer or oleate. In the negative control experiment (buffer control), buffer alone was perfused into the proximal and distal halves of the gut. To exclude a nonspecific accelerating effect of the antagonist, the effect of naloxone with buffer in either the proximal or distal half of the gut was also tested.

Measurement of intestinal transit. Sixty minutes after the start of the perfusion (to allow time for full activation of inhibitory feedback) (22), ~20 µCi 99mTc-diethylenetriamine pentaacetic acid (99mTc-DTPA) (7) was delivered as a bolus into the test segment to begin measurement of intestinal transit (18). Intestinal transit across the proximal half of the gut (150 cm length) was measured by tracking the radioactivity of 3-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 (18, 35) and the radioactivity of the recovered fistulous output were all measured in a gamma well counter. After correcting all counts to time 0, intestinal transit was calculated as the cumulative percent recovery of the delivered 99mTc-DTPA over the collection period (22).

Analysis of data. Intestinal transit results were compared as the cumulative percent recovery of 99mTc over 30 min. To test for the fat-induced ileal brake, the effect of buffer or fat in the distal gut on intestinal transit was compared by paired t-test. To test for the response to naloxone, the effect of the perfusion conditions was compared using one-way repeated measures ANOVA. The computer program used was BMDP Statistical Software (8).

RESULTS

Fat-induced ileal brake. Intestinal transit as represented by the cumulative percent recovery of radioactive marker over 30 min is shown in Figs. 1, 2, and 3. In four dogs, intestinal transit was slowed by oleate in the distal half of the gut as the fat-induced ileal brake with marker recovery decreasing from 74.85 ± 5.6% with buffer alone (buffer control) to 34.6 ± 4.8% with oleate in the distal half of the gut (ileal brake) (P < 0.01) (Fig. 1).

Validation experiment to exclude nonspecific effect. In four dogs, naloxone delivered with buffer into the proximal or distal half of the gut had no significant effect on intestinal transit. Specifically, there was no significant effect of the perfusion conditions because the marker recovery was 74.85 ± 5.6% with buffer alone, 79.0 ± 5.7% with naloxone added to buffer in the proximal gut and 74.9 ± 7.1% with naloxone added to buffer in the distal gut (Fig. 2).

Effect of naloxone in afferent vs. efferent limb of ileal brake. In 11 dogs, during fat-induced ileal brake, intestinal transit without naloxone (ileal brake) or with naloxone delivered into the proximal or distal half of the gut is compared in Fig. 3. Intestinal transit depended on the perfusion condition (P < 0.00001). Without naloxone, the cumulative marker recovery was low at 35.7 ± 7.4% reflecting the triggering of the fat-induced ileal brake (22). However, intestinal transit was accelerated when naloxone was delivered into the
DISCUSSION

The present study confirms previous observations (2, 19) that opioid pathways are involved in the feedback regulation of gastrointestinal transit by ileal fat. In this study, we extended this understanding by reporting the novel observation that the slowing effect of fat in the distal half of the gut on transit through the proximal small intestine was blocked when naloxone was delivered into the proximal half of the gut but not the distal half of the gut. Naloxone was administered into both compartments of the gut, but the reversal of the fat-induced ileal brake only occurred in one compartment. Therefore, we can only conclude that naloxone was acting on a peripherally (gut) located, opioid pathway in the proximal gut (afferent limb). This effect was not due to a nonspecific accelerating property of the opioid antagonist, since intestinal transit was unchanged when naloxone was mixed with buffer.

The lack of an effect of naloxone in the distal gut cannot simply be explained by an inactivation of the opioid antagonist by oleate. In an earlier study, we (34) found that fat-induced jejunal brake was also reversed by naloxone. In that experiment, 60 mM oleate and naloxone (0, 3, 6, and 12 mg) were delivered together into the proximal half of the gut. Because naloxone in the proximal gut was able to reverse the slowing of intestinal transit whether it was coperfused with fat (in the jejunal brake experiment) or buffer (in the ileal brake experiment), a naloxone-oleate chemical effect could not be the explanation for the finding in our study. In the ileal brake experiment, the effenter limb (proximal gut) was separated from the afferent limb (distal gut), but in the jejunal brake experiment, the effenter and afferent limbs were both located in the proximal gut. Therefore, the common feature of both experiments was that naloxone was available to an opioid pathway located in the effenter limb of the response.

Our experiments were performed in a unique animal model equipped with duodenal and midgut fistulas that allowed for compartmental perfusion of naloxone into either the proximal or distal half of the gut. An advantage of this approach over an in vitro model was that the full feedback control circuit was preserved for testing. In addition, unlike the study of Kinsmen and Read (19) using intravenous naloxone, we were able to identify the location of the opioid pathway involved in the response to fat by compartmentalizing luminal naloxone to either the distal (afferent limb) or proximal (effenter limb) half of the gut. Because orally administered naloxone has little systemic effect due to its extensive first-pass biotransformation (6, 20) naloxone administered luminally may act specifically on peripheral (gut) opioid pathways. Thus our region-specific observation with luminal naloxone suggests that a peripheral opioid pathway located on the effenter limb of the response is involved in the slowing of intestinal transit by fat in the distal gut.

Consistent with the published literature (9, 27, 29), we have used the term ileal brake to refer to the response to nutrients delivered into the distal half of the gut. In our experimental model, oleate perfused beyond the midgut fistula had access to the distal half of the small intestine as well as the colon. However, the extent of the role of the colon in the feedback regulation of intestinal transit by fat is unknown.
Our study extends previous reports in humans (19) and rats (2) showing a role for an opioid pathway in the fat-induced ileal brake. Although these studies used intravenous naloxone, the importance of the effect of opioid peptides on the gut has been shown in studies demonstrating that opioid peptides acted directly on circular smooth muscle to increase nonpropulsive contractions (4) and on longitudinal smooth muscles to induce tonic contractions (26).

Opioid peptides also affect nerves of the gut because opioid receptors are found on nerves located in the deep muscular plexus and myenteric plexus (1). Opioid pathways of the enteric nervous system have also been activated by electric field stimulation in canine small intestine (11) and are involved in the peristaltic reflex. Exogenous opiates inhibit the release of ACh (12, 33) and substance P (15) from the excitatory motoneurons responsible for ascending contraction. Opioid peptides also inhibit the release of vasoactive inhibitory peptide and nitric oxide (13) from inhibitory motoneurons responsible for descending relaxation (17).

The exact mechanism whereby a naloxone-blockable opioid pathway on the efferent limb of the fat-induced ileal brake is linked to fat on the afferent limb of the response (distal gut) is unknown. A peptide or neural pathway on the efferent limb of the fat-induced ileal brake is linked to fat on the afferent limb of the fat-induced ileal brake (17).

The regulation of the ileal brake is complex, involving a number of different gut peptides and neural pathways. To date, special attention has centered on peptide YY (PYY), a gut hormone that is localized in the ileum and colon (16). Using the technique of peptide immunoneutralization, we have demonstrated that the slowing of intestinal transit by oleate in the distal half of the gut was reversed by PYY antibody (24). Other pathways involved in the regulation of intestinal transit include 5-hydroxytryptamine (2, 3) and glucagon-like peptide-1 as well (32).

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REFERENCES


