Serotonin and cholecystokinin mediate nutrient-induced segmentation in guinea pig small intestine

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Ellis M, Chambers JD, Gwynne RM, Bornstein JC. Serotonin and cholecystokinin mediate nutrient-induced segmentation in guinea pig small intestine. Am J Physiol Gastrointest Liver Physiol 304: G749–G761, 2013. First published February 7, 2013; doi:10.1152/ajpgi.00358.2012.—Segmentation is an important process in nutrient mixing and absorption; however, the mechanisms underlying this motility pattern are poorly understood. Segmentation can be induced by luminal perfusion of fatty acid in guinea pig small intestine in vitro and mimicked by the serotonin (5-HT) reuptake inhibitor fluoxetine (300 nM) and by cholecystokinin (CCK). Serotoninergic and CCK-related mechanisms underlying nutrient-induced segmentation were investigated using selective 5-HT and CCK receptor antagonists on isolated segments of small intestine luminally perfused with 1 mM decanoic acid. Motility patterns were analyzed using video imaging and spatiotemporal maps. Segmenting activity mediated by decanoic acid was depressed following luminal application of the 5-HT receptor antagonists granisetron (5-HT3, 1 μM) and SB-207266 (5-HT4, 10 nM) and the CCK receptor antagonists devazepide (CCK-1, 300 nM) and L-365260 (CCK-2, 300 nM), but these antagonists did not further depress segmentation when combined. The P2 receptor antagonist pyridoxal phosphate-6-azophenyl-2′,4′-disulfonate (10 μM) had no effect on activity. Serosal application of 5-HT antagonists had little effect on segmentation in the duodenum but reduced activity in the jejunum when granisetron and SB-207266 were applied together. These results reveal that 5-HT3 and 5-HT4 receptors, as well as CCK-1 and CCK-2 receptors, are critical in regulating decanoic acid-induced segmentation. Computational simulation indicated that these data are consistent with decanoic acid activating two pathways in the mucosa that converge within the enteric neural circuitry, while contraction-induced release of 5-HT from the mucosa provides feedback into the neural circuit to set the time course of the overall contractile activity.

Intestinal motility; decanoic acid; segmentation; serotonin; 5-HT3 receptors; 5-HT4 receptors; CCK-1 receptors; CCK-2 receptors

Digestion and mixing of intestinal contents are accomplished through a complex, yet poorly understood, pattern of motility, called segmentation (11). Segmentation is a major feature of the small intestine in the “fed state” after a meal and comprises rhythmic local constrictions in the intestinal smooth muscle that are stationary or propagate only a short distance and occur as isolated bursts of motor activity. Despite its importance, the mechanisms controlling segmentation are yet to be determined.

Fed-state motor activity consists of a mixture of propulsive and segmenting contractions, with the predominant type of contraction being determined, in part, by the nutrient composition of the meal. For example, nonnutritive meals produce predominantly propulsive contractile activity (propagating contractions, which propel content along the intestine) in the duodenum and jejunum of conscious dogs in vivo, while nutritive meals lead to an increase in the proportion of nonpropagating (stationary) contractions and contractions that propagate only a short distance (36). This suggests that propulsion is predominantly triggered by mechanical stimuli arising from an inert meal, that is, distension of the intestinal wall and mechanical distortion of the mucosa, while segmentation results from chemical stimuli probably acting at the level of the mucosa. Interestingly, agents that increase endogenous serotonin (5-HT) levels or mimic the action of 5-HT can increase the proportion of propulsive contractions after a nutrient meal (39), while intravenous administration of neurotensin, somatostatin, secretin, and enkephalin increased the proportion of segmenting contractions after a nonnutritive meal. In contrast, cholecystokinin (CCK) increased propulsive contractions when administered via the same route after a nutrient meal. More recently, we found that fed-state motor activity, including segmenting and propulsive contractions, can be evoked in isolated guinea pig duodenum and jejunum by intraluminal fatty acid and some amino acids (22–24). Segmentation occurs in the absence of distension or mechanical distortion of the mucosa that would result from movement of a chemically inert viscous meal (36). This nutrient-induced motility depends on activation of the enteric neural circuitry and appears to be independent of the intrinsic intestinal pacemakers (23) that may regulate segmenting activity in the mouse small intestine (42). Segmenting and propagating contractions involve activation of complex neural circuits that produce distinct stereotyped motor patterns, i.e., motor pattern generators (22, 23). However, while activities of these pattern generators are enhanced by intraluminal nutrient and contraction types are intermingled in the fed state, there is evidence that they are independently regulated in vitro. For example, luminal cholera toxin enhances propulsive contractile activity but suppresses nutrient-induced segmentation in guinea pig jejunum (17). Blocking intermediate-conductance K+ channels reduces nutrient-induced segmentation but not propulsive activity in the same preparation (13).

The mechanisms coupling nutrients in the lumen to enteric neural activity remain unclear. However, recent results from studies of local reflexes in guinea pig jejunum provide some clues. In particular, local application of 5-HT to the intestinal mucosa was found to evoke an initial inhibitory junction potential (IJP) followed, in some cases, by a more prolonged depolarization, often triggering action potentials in nearby circular muscle (23). This mimicked the effects of local application of 1-phenylalanine, and similar responses were seen with local application of CCK. These results suggest that 5-HT and/or CCK activate excitatory pathways supplying the circular muscle, and as both can be released from mucosal enteroendocrine (EE) cells by different nutrients (22, 24, 26), they are
prime candidates for sensory intermediates coupling luminal nutrients to fed-state motor activity.

The aim of the present study was to investigate the role of mucosal 5-HT and CCK in the motor activity induced by luminal decanoic acid in isolated guinea pig duodenum and jejunum. We tested whether endogenous 5-HT released from the mucosa induces the fed-state activity by luminal infusion with the specific serotonin reuptake inhibitor (SSRI) fluoxetine. Luminal application of antagonists to 5-HT\textsubscript{3} and 5-HT\textsubscript{4} receptors and CCK-1 and CCK-2 receptors was used to assess the contribution of mucosally released 5-HT and/or CCK to responses evoked by decanoic acid. Segmenting and propulsive activities were analyzed separately to determine if their respective pattern generators were differentially regulated by these stimuli. We previously proposed that the bursts of contractile activity characteristic of the fed state in vitro depend on contraction-induced release of 5-HT from the mucosa (12, 13), and we used a computer model simulating the time course of the fed-state motor activity to test this idea in the context of our results. The results indicate that 5-HT and CCK are involved in coupling luminal fatty acid to motor activity and are consistent with mucosal 5-HT also playing a key role in the contraction-induced feedback needed to produce the pattern of alternate contractile and quiescent periods characteristic of this activity.

**METHODS**

**Tissue preparation.** Guinea pigs of either sex (200–400 g) were humanely killed by stunning with a blow to the head and the carotid arteries and spinal cord were severed, as approved by the University of Melbourne Animal Experimentation Ethics Committee. The abdominal cavity was opened, and 5 to 7-cm segments of intestine were dissected. The duodenum was taken from the gastroduodenal junction, while the proximal jejunum was dissected 5 cm anal from the duodenojejunal loop. Each segment was flushed clean, placed in an organ bath superfused with bubbled physiological saline (37°C, 5 mL/min, 95% O\textsubscript{2}, 5% CO\textsubscript{2}), and lined with silicone elastomer (Sylgard, compound 184, Dow Corning) and with a black background to maximize contrast. Cannulas were inserted into each end of the intestinal segments and secured with nylon thread. The oral cannula, connected to an adjustable reservoir filled with physiological saline, was used to change luminal pressure and influence antagonists. The anal cannula, connected via a three-way stopcock to a vertical outflow tube, was used to adjust backpressure and maintain luminal pressure.

**Experimental protocols.** Isolated intestinal segments were equilibrated for 30 min at resting luminal pressure (1–3 cmH\textsubscript{2}O), and spontaneous motor activity was monitored. Tissue integrity was assessed by raising luminal pressure to threshold until persistent propulsive contractions were evoked (24). Spontaneous motor activity ceased after three threshold measurements. The lumen of each tissue segment was then flushed with physiological saline (20 ml) containing 300 nM fluoxetine or 1 mM decanoic acid to produce fed-state motility, and contractile activity was recorded at resting pressure for the duration of the experiment, as described previously (12, 22, 24). In most cases, when antagonists were used, they were added to the luminal infusion reservoir with fluoxetine or decanoic acid and, therefore, were present in the lumen throughout the recording period. Recordings made with saline in the lumen served as the time controls. In a small group of experiments, antagonists were added to the superfusing solution at the start of the experiment as serosal application, and again the antagonists were present throughout the recording period.

**Image acquisition and analysis of spatiotemporal maps.** Video recordings (20 min, 30 frames/s, 640 × 840 resolution) of intestinal movements were captured using a Logitech Quickcam pro 9000 camera and Virtual Dub 9.8 software. Recordings were processed using edge-detection software and visualized as spatiotemporal maps with MATLAB (version 2011a), as described previously (24). Maps were analyzed by measuring total number (frequency) and contraction type. Contractions were defined as propulsive or segmenting: propulsive contractions appeared as oral constrictions that propagated the length of the tissue segment, while segmenting contractions appeared as highly localized contractions that did not propagate more than half the length of the tissue segment in either direction and, in many cases, did not propagate at all (24). Other parameters measured included latency, duration of episodic activity (burst duration), and intervals between episodes of activity (quiescence). Mean contraction numbers for each contraction type were calculated from 6–16 preparations and analyzed further, as they represented the most sensitive indicator of activity.

**Drugs.** To test for the involvement of 5-HT, CCK, and P2X receptors in mediating segmentation, fluoxetine, CCK peptide fragment 26–33 (CCK-8), and pyridoxal phosphate-6-azophenyl-2′,4′-disulfonate (PPADS; Sigma Aldrich), SB-207266 [N-[1-(butyl-4-pipеридинил)метил]-3,4-дихидро-2Н-[1,3]-оксазин[3,2-а]индоле-10-карбоксамид], granisetron (kindly supplied by SmithKline Beecham Pharmaceuticals, Middlesex, UK), tropisetron (Sandor Pharma), and devazepide and L-365260 (gifts from ML Laboratories) were applied to the lumen or in the bath. Decanoic acid was dissolved in a 1:1 solution of absolute ethanol and then diluted to a 100 mM stock solution in distilled water. All other drugs were made up in distilled water as stock solutions and diluted in saline to achieve final volume for perfusion.

**Mathematical model of segmentation activity.** We used a previously published abstract model of bursting activity (13) to investigate how 5-HT and CCK receptor antagonists regulate segmentation activity. The model describes activity in populations of the intrinsic sensory neurons (ISNs), excitatory motor neurons, and inhibitory motor neurons and in the circular muscle (a more complex model incorporating interneurones produces the same results). The ISNs receive a small constant input to represent the nutrient stimulus in the lumen. The resulting activity is then transmitted to the excitatory and inhibitory motor neurons, where transmission to excitatory motor neurons has a fast time course and transmission to inhibitory motor neurons has a slow time course. In this model, the circular muscle does not produce individual contractions. Instead, it produces periods of time during which individual contractions can readily occur (activity episodes) because of the differences in activity in the excitatory and inhibitory motor neurons. Activity episodes in the circular muscle produce excitatory feedback to the ISNs with a time course matching contraction-induced release of 5-HT from the mucosa. A copy of the model can be obtained from ModelDB or by contacting the authors (13).

**Statistics.** Values are means ± SE. Statistical comparisons were made using a two-way ANOVA, with post hoc Bonferroni’s tests for individual significance, unless otherwise stated. Latencies were compared using a two-tailed Mann-Whitney test with 95% confidence intervals. Significance was reflected by $P < 0.05$.

**RESULTS**

**Controls and spontaneous motor activity.** Contractions were occasionally seen at resting luminal pressure in the duodenum (3 cmH\textsubscript{2}O, $n = 179$) and jejunum (2 cmH\textsubscript{2}O, $n = 193$) during equilibration. Spontaneous activity varied in magnitude between experiments but was not different between tissues and ceased after threshold measurements (Fig. 1A). Time controls revealed minimal spontaneous activity in the presence of physiological saline alone. On average, the duodenum ($n = 9$) produced $5 ± 2$ contractions per 20-min recording (0.25 ± 0.13 min\textsuperscript{-1}). There was no significant difference in the fre-
Frequency of contractions compared with the jejunum (n/H11005 14, 0.21 ± 0.11 min/H11006 0.11 min/H11002 1). These contractions were classified as propulsive or segmenting (for definitions see METHODS), because earlier studies indicate that they are regulated by distinct motor pattern generators (13, 17). Propulsive (0.10 ± 0.05 and 0.11 ± 0.07 min/H11002 1 in duodenum and jejunum, respectively) or segmenting (0.13 ± 0.09 and 0.09 ± 0.06 min/H11002 1 in duodenum and jejunum, respectively) contractions did not differ in frequency between tissues.

Intraluminal infusion of fluoxetine evokes segmentation. Luminal perfusion with the SSRI fluoxetine (300 nM; Fig. 1B) evoked activity similar to the response induced by luminal perfusion with the fatty acid nutrient decanoic acid (17, 24) (Fig. 1B). Similar activity was also seen in the presence of luminal 5-HT (30 nM; D) or the 5-HT4 receptor agonist RS-67506 (100 μM; E) and in guinea pig jejunum in the presence of CCK-8 (30 nM; F). Dark vertical band in B is caused by tissue properties (i.e., gland) creating an artificial expansion of gut diameter.

Intraluminal infusion of fluoxetine evokes segmentation. Luminal perfusion with the SSRI fluoxetine (300 nM; Fig. 1B) evoked activity similar to the response induced by luminal perfusion with the fatty acid nutrient decanoic acid (17, 24) (Fig. 1B). Similar activity was also seen in the presence of luminal 5-HT (30 nM; Fig. 1D) or with the 5-HT4 receptor agonist RS-67506 (100 μM; Fig. 1E). On average, the contraction frequency in the duodenum (n = 13) was 2.3 ± 0.83 min⁻¹, a 900% increase compared with controls. Propulsive contractions were infrequent (0.1 ± 0.04 min⁻¹) and did not differ significantly from those in time controls. Thus the increase in overall activity was due to a large increase in segmenting contractions (2.2 ± 0.85 min⁻¹). Similar levels of motor activity were observed in the jejunum (n = 12, 2.16 ± 0.85, 0.16 ± 0.038, and 2.05 ± 0.8 min⁻¹ for total, propulsive, and segmenting contractions, respectively). The latency for initiation of fluoxetine-evoked activity was similar in each region, and once activity began, it occurred in episodic bursts throughout the recording period. There were no significant differences between tissues in other contractile properties (Table 1).

Luminal 5-HT receptor antagonists depress fluoxetine-induced motility. Addition of selective 5-HT3 or 5-HT4 receptor antagonists to the lumen reduced fluoxetine-evoked motility in the duodenum or jejunum to levels only marginally greater than those in the time controls. This was due to a reduction in segmenting contractions, as propulsive contractions were largely unaffected.

In the duodenum, the total number of contractions was reduced to 18% of the original fluoxetine response when the 5-HT3 receptor antagonist granisetron (n = 12, 1 μM) was perfused together with fluoxetine in the lumen (Fig. 2D, Table 2). This was due to a significant reduction in segmenting contractions to 27% of the fluoxetine response (0.56 ± 0.19 min⁻¹, P = 0.0005; Fig. 2F), while propulsive contractions showed an insignificant increase (Fig. 2E). The 5-HT4 receptor antagonist SB-207266 (n = 10, 10 nM) reduced overall contractions to 35% of fluoxetine activity, again due to a marked reduction in segmenting contractions to 13% of the response to fluoxetine (0.28 ± 0.15 min⁻¹). Granisetron + SB-207266 (n = 10) was no more effective than SB-207266 alone, and the combined
5-HT_{3}/5-HT_{4} receptor antagonist tropisetron had a similar effect (10 \mu M, n = 10). This was due to a decrease to only 10–20% of segmenting contractions compared with the response to fluoxetine (0.35 ± 0.18 and 0.22 ± 0.08 min^{-1} for granisetron + SB-207266 and tropisetron, respectively). Comparisons between time controls and frequencies of segmenting contractions in the presence of the antagonists (Table 2) show that, in all cases, segmenting contractions were more frequent in the presence of fluoxetine and one or more 5-HT receptor antagonists than in the time controls. However, this difference was small and not always statistically significant, suggesting that 5-HT_{3} and 5-HT_{4} receptor antagonists effectively abolished segmentation produced by fluoxetine.

Similar results were obtained in the jejunum, where fluoxetine-induced activity was reduced to 27% of the original increase by granisetron (n = 14) or SB-207266 (n = 10; Fig. 2, A–D and G, Table 2). Segmenting contractions fell to 20% of the response to fluoxetine [0.4 ± 0.16 min^{-1} by granisetron (P = 0.0041) and 0.31 ± 0.15 min^{-1} by SB-207266; Fig. 2].

In the duodenum, no significant differences in propulsive contractions were observed. Granisetron + SB-207266 did not further depress activity, and this was due to a reduction in segmentation alone (n = 13, 0.4 ± 0.16 min^{-1}, P < 0.0048).

Again, luminal perfusion with tropisetron had an effect similar to the effect of the combined antagonists [n = 10, 0.48 ± 0.19 min^{-1} total contraction and 0.35 ± 0.16 min^{-1} segmenting contraction (P = 0.0341)].

Does 5-HT have a role in nutrient-induced motility in vitro? The results with fluoxetine strongly suggest that mucosally released 5-HT induces segmenting contractions in guinea pig duodenum and jejunum. Accordingly, we undertook another set of experiments in which the 10-carbon fatty acid decanoic acid (1 mM) was luminally perfused into the duodenum and jejunum. At 1 mM, decanoic acid has previously been shown to evoke episodes of contractile activity, including segmenting contractions, alternating with periods of quiescence in the duodenum and jejunum (12, 16, 23). In our study, infusion of decanoic acid into the lumen increased overall contractile activity dramatically in the duodenum (from 0.25 ± 0.11 min^{-1} with saline to 3.45 ± 0.77 min^{-1}) and jejunum (from 0.21 ± 0.11 min^{-1} with saline to 4.23 ± 1.08 min^{-1}, Table 3).

There was no significant difference between the two tissue regions in total number of contractions [3.45 ± 0.77 and 4.23 ± 1.08 min^{-1} for duodenum (n = 16) and jejunum (n = 14), respectively], propulsive contractions (0.63 ± 0.23 and 1.02 ± 0.46 min^{-1} for duodenum and jejunum, respectively), or segmenting (2.82 ± 0.66 and 3.21 ± 0.95 min^{-1} for duodenum and jejunum, respectively) contractions. In contrast to the effects of fluoxetine, decanoic acid significantly increased propulsive contractions in the duodenum (P = 0.001) and jejunum (P = 0.0005). However, the major increase in contractile activity was clearly due to increased segmentation.

There were no tissue differences in latency for initiating decanoic acid-evoked activity, but latency was increased compared with fluoxetine-evoked activity in the duodenum (P = 0.03). Interestingly, both tissues showed greater episodic activity and shorter quiescent periods in the presence of luminal decanoic acid than fluoxetine (duodenum: P = not significant for burst activity, P = 0.003 for quiescence, P < 0.05 at 75 min; jejunum: P = 0.019 for burst activity, P = 0.001 for quiescence, P = 0.0004 for time effect; Table 1).

Luminal 5-HT receptor antagonists depress decanoic acid-induced motility. Decanoic acid-induced contractile activity was reduced, but not abolished, in the duodenum and jejunum when granisetron or SB-207266 was infused with decanoic acid into the lumen (Fig. 3). This was due to a large reduction in segmentation contractions, although propulsive contractions were reduced to just above control levels by granisetron in the duodenum and to the levels of the time controls by granisetron in the jejunum. SB-207266 reduced propulsive contractions to control levels in both regions.

In the duodenum, luminal granisetron reduced overall activity to ~22% of the decanoic acid response (n = 10, P < 0.0005). This change was due to halving of propulsive contractions and a reduction to 30% of segmenting contractions compared with the response to decanoic acid alone (Table 3). Luminal perfusion with SB-207266 (n = 6) reduced overall activity to 25% compared with nutrient alone and reduced propulsive and segmenting contractions to 36% and 26% of the decanoic acid-induced activity, respectively. Granisetron and SB-207266 (n = 9) did not reduce activity further, and their combined effect was indistinguishable from that of luminally perfused tropisetron (Fig. 3, A–C). Combined blockade of 5-HT_{3} and 5-HT_{4} receptors with granisetron + SB-207266 or with tropisetron (n = 8) depressed propulsive contractions evoked by decanoic acid to less than one-third of the decanoic acid-induced increase and segmenting contractions to 15–22% of the decanoic acid-induced increase and 50% of the original increase.

In the jejunum, decanoic acid-evoked motility was reduced to levels similar to those in the duodenum (19–26%) with luminal granisetron (n = 10) or SB-207266 (n = 10; Fig. 3, D–F, Table 3). Decanoic acid-induced propulsive contractions were effectively abolished by granisetron or SB-207266. Segmenting contractions were reduced to 20% of those with

Table 1. Properties of segmentation activity in guinea pig small intestine in response to luminal infusion of fluoxetine and decanoic acid

<table>
<thead>
<tr>
<th></th>
<th>Duodenum</th>
<th>Jejunum</th>
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<tr>
<td></td>
<td>Fluoxetine</td>
<td>Decanoic acid</td>
</tr>
<tr>
<td>Sample size</td>
<td>13</td>
<td>16</td>
</tr>
<tr>
<td>Latency, min</td>
<td>9.1 ± 1.1</td>
<td>12.8 ± 1.0</td>
</tr>
<tr>
<td>Burst duration, s</td>
<td>25.6 ± 3.3</td>
<td>82.1 ± 43.2</td>
</tr>
<tr>
<td>Quiescence, min</td>
<td>5.5 ± 1.42</td>
<td>2.97 ± 0.8</td>
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Values are means ± SE. Note no difference between duodenum and jejunum for latency, burst activity, or quiescence within treatment for fluoxetine or decanoic acid. Latency increased in decanoic acid vs. fluoxetine. Burst activity also increased with decanoic acid vs. fluoxetine alone, but this was only significant in the jejunum. Quiescence decreased for both tissues when comparing luminal stimuli. Quiescence was reduced in the duodenum and jejunum in a time-dependent manner. *P < 0.05.
decanoic acid alone by luminal granisetron; SB-207266 decreased segmenting contractions to 32% (Fig. 3). Granisetron/SB-207266 did not yield a further reduction in the response ($n=10$; Fig. 3). The effect of tropisetron ($n=8$) was similar to that of the combined specific antagonists.

**Serosal application of 5-HT receptor antagonists on decanoic acid-induced motility.** The route of application of 5-HT$_3$ and 5-HT$_4$ receptor antagonists may alter their efficacy on motility pathways (43), with results from bath application (i.e., via the serosal surface) different from results from luminal application. Accordingly, we tested the effects of adding granisetron and SB-207266 to the organ bath on decanoic acid-induced motility. The results are shown in Fig. 4.

There was a significant increase in motility in the duodenum compared with the response to decanoic acid alone with bath-applied granisetron ($n=7$, $P<0.0001$), and this was due to increases in propulsive and segmenting (both $P<0.0001$) contractions. On the other hand, SB-207266 produced a small decrease in overall (13% reduction), propulsive (23% reduction), and segmenting (7% reduction) contractions. When the antagonists were combined, the effects were very similar to the effect of SB-207266 alone (Fig. 4, Table 3).
In the jejunum, total contractions increased with serosal granisetron or SB-207266 compared with the response to decanoic acid [Fig. 4D, Table 3; 5.72 ± 1.58 min⁻¹ with granisetron (n = 6, P < 0.0001) and 7.02 ± 3.26 min⁻¹ with SB-207266 (n = 8, P < 0.0001)]. This was due to an increase in segmenting contractions (Fig. 4F) compared with the response to decanoic acid alone [5.00 ± 1.41 min⁻¹ for granisetron segmenting (P < 0.0001)] and 5.95 ± 3.11 min⁻¹ for SB-207266 segmenting (P < 0.0001)], with only small changes in propulsive contractions. However, when combined, these 5-HT₃ and 5-HT₄ receptor antagonists significantly decreased the total number of contractions to levels indistinguishable from control values (n = 10), and this was due to a decline in segmenting contractions (0.86 ± 0.23 min⁻¹, P < 0.0001).

Is CCK involved in decanoic acid-induced motility? Fatty acids in the lumen of the duodenum and jejunum release CCK from EE cells (26), and mucosal application of CCK evokes excitatory junction potentials (EJPs) in the circular muscle of guinea pig jejunum (23). Furthermore, luminal infusion of CCK can produce segmenting motor activity (Fig. 1F). Thus we examined whether blockade of CCK receptors also affects decanoic acid-induced motility patterns.

In the duodenum, overall activity was reduced to 46–55% of the decanoic acid-evoked response with the selective CCK-1 (devazepide, 300 nM, n = 14) and CCK-2 (L-365260, 300 nM, n = 10) receptor antagonists but was not further reduced when the antagonists were combined (Fig. 5A). There was a significant decrease in propulsive contractions, effectively to time control levels (Table 3). Segmenting contractions were reduced to half of the decanoic acid-evoked response [devazepide: 1.90 ± 0.50 min⁻¹ (P < 0.0035), L-365260: 1.93 ± 0.42 min⁻¹ (P = 0.0089), combined: 1.62 ± 0.65 min⁻¹ (P = 0.0033)].

In the jejunum (Fig. 5B), luminal infusion of either antagonist depressed activity. Devazepide (n = 12) and L-365260 (n = 10) reduced overall contractions. In combination, the CCK receptor antagonists appeared more effective at reducing total contractions than either antagonist alone (n = 10). Propulsive contractions decreased to control levels (Table 3). However, while segmenting contractions were significantly reduced with L-365260 (1.60 ± 0.43 min⁻¹, P = 0.0001) and when it was combined with devazepide (1.05 ± 0.31 min⁻¹, P = 0.0001; time effect P = 0.05), the effect of devazepide alone on segmenting contractions failed to reach significance (2.16 ± 0.51 min⁻¹, P = 0.059).

Using a combination of granisetron, SB-207266, devazepide, and L-365260 flushed together with decanoic acid into the intestinal lumen, we tested if CCK and 5-HT act along the same pathway. In the duodenum (n = 8), overall activity was reduced to 54% (2.00 ± 0.83 min⁻¹, P = 0.0024) of the decanoic acid-induced response (Fig. 5A), and propulsive and segmenting contractions were reduced. A similar reduction in motility (to ~30%) was seen in the jejunum (Fig. 5B) due largely to a decrease in segmenting contractions (1.08 ± 0.29 min⁻¹, P < 0.001), while propulsive contractions were reduced to 57% of the decanoic acid-induced activity, although these were still significantly more frequent than in the time controls. The response to decanoic acid was not abolished in either tissue, suggesting the involvement of another, unknown, mediator.

Blocking P2X receptors does not alter decanoic acid-induced motility. The P2 receptor antagonist PPADS depresses local inhibitory reflexes evoked by application of amino acids to the mucosa (23), suggesting that a purine may mediate some effects of nutrients on intestinal motility. However, we found no change in contraction number in the presence of lumen- or bath-applied PPADS (10 μM) in the duodenum or jejunum with decanoic acid-induced contractile activity (luminal: 2.96 ± 1.41 and 2.94 ± 0.67 min⁻¹ in duodenum and jejunum, respectively, both n = 6, P = not significant; bath not shown).

Modeling 5-HT and CCK effects on contractile activity during segmentation. The data described above suggest that 5-HT₃, 5-HT₄, CCK-1, and CCK-2 receptors are involved in generating the motor activity produced by luminal decanoic acid. As the effect of the combined antagonists was no greater than that of the 5-HT₃ or 5-HT₄ receptor antagonist alone, one might conclude that these receptor subtypes act in series, with each having a role at different critical points along a single pathway. However, 5-HT and CCK are contained in, and released by, different populations of EE cells, suggesting that,
Table 3. Mean contraction frequencies in duodenum and jejunum in response to saline control, decanoic acid infusion, and 5-HT, CCK, and P2 receptor antagonists

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<tr>
<th></th>
<th>Duodenum</th>
<th>Jejunum</th>
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<tr>
<td></td>
<td>n</td>
<td>Total</td>
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<tr>
<td>Decanoic acid</td>
<td>16</td>
<td>3.45 ± 0.77</td>
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<td>P</td>
<td>&lt;0.0001</td>
<td>0.001</td>
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**Luminal application of 5-HT receptor antagonists**

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<th></th>
<th>n</th>
<th>Mean ± SE</th>
<th>P</th>
<th>Mean ± SE</th>
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<tr>
<td>Granisetron</td>
<td>10</td>
<td>1.12 ± 0.46</td>
<td>&lt;0.0001 NS</td>
<td>1.08 ± 0.29</td>
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<td>SB-207266</td>
<td>6</td>
<td>0.85 ± 0.37</td>
<td>NS</td>
<td>0.71 ± 0.03</td>
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<td>P</td>
<td></td>
<td>&lt;0.0001 NS</td>
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**Serosal application of 5-HT receptor antagonists**

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<th>Mean ± SE</th>
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<tr>
<td>Granisetron</td>
<td>7</td>
<td>1.28 ± 0.46</td>
<td>&lt;0.0001 NS</td>
<td>1.24 ± 0.44</td>
<td>&lt;0.0001 NS</td>
</tr>
<tr>
<td>SB-207266</td>
<td>6</td>
<td>3.00 ± 0.91</td>
<td>&lt;0.0001 NS</td>
<td>2.86 ± 0.90</td>
<td>&lt;0.0001 NS</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.0001 NS</td>
<td>&lt;0.0001 NS</td>
<td>&lt;0.0001 NS</td>
<td>&lt;0.0001 NS</td>
</tr>
</tbody>
</table>

**Luminal application of CCK receptor antagonists**

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Mean ± SE</th>
<th>P</th>
<th>Mean ± SE</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Devazepide</td>
<td>14</td>
<td>2.10 ± 0.48</td>
<td>&lt;0.0001</td>
<td>2.30 ± 0.53</td>
<td>&lt;0.0001 NS</td>
</tr>
<tr>
<td>L-365260</td>
<td>10</td>
<td>3.03 ± 0.44</td>
<td>0.0004 NS</td>
<td>1.90 ± 0.50</td>
<td>0.38</td>
</tr>
<tr>
<td>P</td>
<td></td>
<td>&lt;0.0001 NS</td>
<td>&lt;0.0001 NS</td>
<td>&lt;0.0001 NS</td>
<td>0.17 ± 0.04</td>
</tr>
</tbody>
</table>

Values (means ± SE) represent frequency of contraction types (contractions/min) for duodenum and jejunum in response to infusion with decanoic acid and together with 5-HT, CCK, and P2 receptor antagonists over total recording period. Combined 5-HT/CCK, granisetron, SB-207266, devazepide, and L-365260. Statistical comparisons (P values) are between saline (control) and nutrient/drug combinations (by 2-way ANOVA).

rather than operating in series, 5-HT and CCK mediate separate pathways that converge within the neural circuitry. We explored this using a reduced computational model that we have shown can account for some of the temporal properties of decanoic acid-induced motor activity (13).

To compare the model’s output with experimental data, the area under the curve of circular muscle activity during an activity episode was divided by the period of the whole oscillation (activity episode and quiescence). This accounted for changes in frequency and amplitude of oscillating activity (Fig. 6A) and is expressed as a percentage of the value obtained from the previously published data.

To simulate blocking CCK receptors, the “nutrient” input to the ISNs was reduced by a set proportion, with the remainder being taken to be due to decanoic acid-induced 5-HT release. In this case, feedback from muscle contraction was unaltered. Blockade of 5-HT receptors was simulated by again reducing nutrient stimulus by a set proportion, but also by proportionately reducing the contraction-induced feedback. Blockade of CCK and 5-HT receptors was assumed to reduce the nutrient stimulus and the feedback. Examples of the types of results are illustrated in Fig. 6.

Reducing the nutrient stimulus without altering feedback from the contracting muscle, i.e., simulating blocking CCK receptors alone, increased the interval between activity episodes without much change in the size and shape of these episodes (Fig. 6A). Thus, in the predicted model, only a relatively small effect on the overall contractile activity was observed until the nutrient stimulus was reduced by >75% (Fig. 6B), when activity was reduced to a low constant level. Thus, unless the great majority of the nutrient-induced activity was due to CCK, the model cannot account for the fact that combined addition of CCK and 5-HT receptor antagonists was no more effective than 5-HT blockade alone.

We tested the effects of reducing the feedback without altering the nutrient stimulus. This led to smaller activity peaks without altering the frequency of the activity episodes (Fig. 6A) and markedly increased the overall sensitivity of the system, so that >50% reductions in feedback led to effective abolition of the activity. This set of conditions simulated a situation in...
which CCK initiated all nutrient-induced contractile activity, while 5-HT was only involved in the feedback. However, this seems an unlikely mechanism, as the intervals between contractions were markedly increased by blocking CCK or 5-HT receptors in the physiological experiments.

Combining a reduction in the nutrient stimulus and a reduction in feedback, simulating blockade of receptors activated by nutrient- and contraction-induced release of 5-HT, produced an even sharper decline in overall contractile activity, with >25% reduction leading to effective abolition (Fig. 6). Under these conditions, further reducing the nutrient-induced stimulation by removing the effect of CCK receptor activation will have no further effect.

Taken together, the modeling suggests that CCK initiates the motor activity, while mucosally released 5-HT plays a much more substantial role in providing contraction-induced feedback to the circuit and a lesser role in initiating segmentation.

**DISCUSSION**

The present study demonstrates the importance of 5-HT and CCK as critical mediators in regulating segmentation in isolated guinea pig small intestine. The role of 5-HT in regulating fluoxetine-evoked motor patterns in the duodenum and jejunum is similar, as this SSRI produces marked increases in segmenting contraction that are effectively abolished by blocking 5-HT3 or 5-HT4 receptors. Nutrient-induced motility is regulated by 5-HT and CCK probably acting within the mucosal layer via 5-HT3 and 5-HT4 receptors and CCK-1 and CCK-2 receptors, respectively. The antagonists affect propulsive and segmenting contractions to varying degrees in either region, and their actions are largely dependent on the route of administration. The failure of combined 5-HT and CCK receptor antagonists to completely abolish activity suggests that other, as yet unidentified, mediators contribute to the nutrient reflex pathways controlling segmentation and propulsive motor patterns, although it is unlikely to be a purine, as PPADS did not affect motility.

**Mechanisms responsible for fluoxetine-induced segmentation.** It is well established that 5-HT regulates motility by activating nerve terminals of intrinsic sensory neurons (4, 8, 9, 15, 17, 23) and that a similar effect is seen with low (nanomolar) concentrations of fluoxetine (28). The present results indicate that fluoxetine-induced motor activity in isolated guinea pig duodenum and jejunum consists virtually exclusively of segmenting contractions that are largely blocked by luminal infusion of 5-HT3 or 5-HT4 receptor antagonists.

Myenteric ISNs almost certainly mediate this 5-HT-induced motility, which appears to be due to a distinct segmentation pattern generator (12, 17), with the pattern generator responsible for propulsion (peristalsis) being less sensitive to mucosally released 5-HT. Terminals of myenteric ISNs contain 5-HT3 receptors and respond to mucosally applied 5-HT (2, 4,
It has also been proposed that 5-HT3 receptors are present on enterochromaffin (EC) cells (2, 21, 34, 38), although this has not been directly established (30). Recent findings by Spencer et al. (40) showed that distension-evoked peristalsis in guinea pig distal colon persists when the mucosa is removed and, therefore, is independent of 5-HT release from EC cells, although whether this is relevant to segmentation in the small intestine remains to be seen. The failure of fluoxetine to enhance propulsive activity is certainly consistent with the findings of Spencer et al., i.e., that mucosal 5-HT plays no role in the initiation of peristalsis (2, 44).

While 5-HT3 receptors have been confirmed in the myenteric plexus, the role and location of 5-HT4 receptors are subjects of debate in the literature (21, 22, 27, 31, 38). The presence of 5-HT4 receptors has been confirmed on submucosal and myenteric ISNs projecting to the mucosa in guinea pig small intestine (33) and may be involved in the regulation of motility (32). Li et al. (25) observed decreased motility in tryptophan hydroxylase (TPH) type 2 (TPH2) knockout mice, which lack the neuronal isoform of TPH2, the rate-limiting enzyme in 5-HT biosynthesis; however, TPH1 knockout mice lacking the nonneuronal isoform expressed in EC cells did not show altered motility. Nevertheless, the simplest mechanism by which luminal fluoxetine can induce segmentation involves the release of endogenous 5-HT from EC cells to excite 5-HT3 and 5-HT4 receptors on myenteric and submucosal sensory nerve terminals to activate the segmentation pattern generator.

The observation that 5-HT3 and 5-HT4 receptors are involved in segmentation provides the first evidence of specific neural mechanisms underlying this reflex and raises questions about the role of 5-HT in segmentation. Granisetron probably acts via blockade of 5-HT3 receptors on the terminals of myenteric ISNs projecting to the basolateral surface of the mucosa. However, the 5-HT4 receptor antagonist SB-207266 effectively blocks segmenting contractions. Both antagonists are equally effective at depressing segmentation but are not additive. This may indicate that the two receptor subtypes operate in series or that one acts in a permissive fashion for the other. Clarification of this issue requires more specific data about localization of these receptor subtypes and their interactions at a membrane level than is currently available.

Mechanisms responsible for decanoic acid-induced segmentation. Segmentation is evoked in guinea pig small intestine by luminal infusion of different nutrients, including fatty acids (24) and amino acids (23); however, the mechanism by which chemical sensing occurs is unclear. Our results show that luminally infused nutrient induced a robust segmenting motor pattern involving 5-HT and CCK. We previously identified distinct local reflexes evoked by amino acids applied to the mucosa, which were mediated via 5-HT3, 5-HT4, and P2X

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**Fig. 4.** Effects on motor activity induced by decanoic acid and serosal 5-HT receptor antagonists in duodenum and jejunum. Time course of total (A and D), propulsive (B and E), and segmenting (C and F) contractions in response to luminally applied decanoic acid and serosal 5-HT3 or 5-HT4 receptor antagonists. Decanoic acid-induced motility was significantly increased in duodenum in response to bath-applied granisetron and slightly reduced by SB-207266 but increased over time (A–C). In jejunum, contractile activity increased with granisetron or SB-207266. In the presence of granisetron + SB-207266, frequency of segmenting contractions was reduced in a time-dependent manner (D and F) to levels very close to saline control. Values are means ± SE (n > 6). P < 0.05.
receptors presumably located on the terminals of ISNs (24). In other experiments, luminal infusion of decanoic acid produced segmentation activity characterized by EJPs recorded at the site of isolated circular muscle contractions, which were time-locked to IJPs in the surrounding circular muscle (23). These results indicate that amino acids evoke local reflexes contributing to pathways controlling segmentation motor activity and suggest that decanoic acid may operate along a similar pathway. CCK receptors are not involved in amino acid-evoked inhibitory reflexes but do appear to mediate excitatory reflexes (22), and our results indicate that 5-HT and CCK receptors are essential for fatty acid-induced segmentation.

Fatty acids behave as endogenous ligands to G protein-coupled receptors (GPRs), including those that recognize medium- and long-chain fatty acids, such as GPR40–43 and GPR120 (7, 14). Sykaras et al. (43) recently demonstrated mRNA expression for the fatty acid receptors GPR40/FFAR1, GPR120/O3FAR1, GPR41/FFAR3, and GPR43/FFAR2 in mouse duodenal I cells, EE cells that release CCK, highlighting a critical role for these cells in nutrient sensing. Similar mechanisms relating to nutrient sensing along the gut-brain axis have been suggested (35). Whether decanoic acid activates all these different classes of fatty acid receptor is not established, and it is possible that other longer-chain fatty acids may have other effects on motor activity by activating a different subset of fatty acid receptors. Nevertheless, our study demonstrates that luminal infusion of decanoic acid evokes propulsive and segmenting contractions via release of 5-HT and CCK and that specific 5-HT3 and 5-HT4 and CCK-1 and CCK-2 receptor antagonists block this release, as would be predicted from the known distributions of the fatty acid receptors. Whether decanoic acid is a component of the contents of the jejunum after a meal is unknown, but the receptors on which it may act have a wide sensitivity to different fatty acids and would be expected to initiate motility changes with any of their normal ligands. Thus we can reasonably assume that the pharmacological profile of the motility effects of decanoic acid is representative of that of longer-chain fatty acids as well.

Effect of 5-HT3 and 5-HT4 receptor antagonists on decanoic acid-induced segmentation. The current study confirms the importance of 5-HT as a mediator of decanoic acid-induced segmentation (25). Luminal infusion of granisetron, SB-207266, or tropisetron effectively reduced, but did not abolish, segmentation contractions. These findings are consistent with the effects of these antagonists on segmentation induced by luminal fluoroxetine and provide further support for the idea that segmentation can be initiated via activation of 5-HT3 and 5-HT4 receptors. The results also suggest that neurons expressing 5-HT3 and 5-HT4 receptors may converge along the excitatory limb of the same reflex pathway. Many ISNs also appear to function as interneurons integrating and relaying feedback to other sensory neurons along the same reflex pathway (5, 18). This behavior probably involves ISNs expressing 5-HT3 and 5-HT4 receptors in their mucosal terminals, as well as ISNs sensitive to other mediators, as segmentation contractions persist despite combined blockade.

In contrast to luminal infusion, the effects of bath-applied 5-HT receptor antagonists on decanoic acid-induced segmentation differed between the duodenum and the jejunum. The 5-HT4 receptor antagonist enhanced contractile activity in both regions, while the 5-HT4 receptor antagonist produced small decreases in contractile activity in the former and a significant increase in the latter. When combined, the antagonists were no more effective than the 5-HT4 receptor antagonist alone in the duodenum but produced a significant reduction in activity in the jejunum, despite the enhanced segmentation with each antagonist alone. The different effects between tissues and application of antagonists presumably reflects variations in the accessibility of receptors at different locations (mucosa vs. myenteric plexus, duodenum vs. jejunum), which may differentially affect the control of segmentation and requires further investigation.

The differences between effects of antagonists produced by routes of administration raise the possibility of roles for neuronal 5-HT in regulation of segmenting and propulsive motor patterns. Serosal application of antagonists would be expected to preferentially affect synapses in the myenteric plexus, in contrast to luminal application, which would preferentially act...
on receptors via the mucosa. Thus the increased segmenting activity produced by serosal granisetron is presumably due to blocking an inhibitory pathway depending on at least one synapse employing 5-HT3-mediated transmission (17). Unfortunately, while the computational model that we used in this study can discriminate the effects of large changes in the role of fast and slow excitatory synaptic potentials, it lacks the resolution to determine the effects of more subtle changes, e.g., those due to blocking 5-HT3 or 5-HT4 receptors (13). To address this, we would need data including the specific neurons that have 5-HT3 receptor-mediated fast excitatory postsynaptic potentials and the specific functional classes of nerve terminals expressing 5-HT4 receptors to produce presynaptic inhibition and a significantly more realistic model.

**Effects of CCK-1 and CCK-2 receptor antagonists on decanoic acid-induced segmentation.** CCK receptors are found throughout the gastrointestinal tract (15) and regulate motility and digestion of protein and fat (29, 37). In the present study, decanoic acid-induced segmentation appears to be mediated, in part, by the release of CCK and its action on CCK-1 and CCK-2 receptors. This is consistent with previous studies using endocrine cell lines, which confirm that decanoic acid and other fatty acids evoke the release of CCK (26, 29). The precise location of CCK receptors in the mucosa has not been identified in the guinea pig, but nerve terminals containing CCK receptors have been localized on duodenal I cells (26), in both enteric plexuses in the rat (41), and in guinea pig ileum (37). Our results suggest that CCK is released from mucosal EE cells in a similar way to decanoic acid stimulation of 5-HT release to activate segmentation motor patterns.

**Effect of P2X receptor antagonist on decanoic acid-induced segmentation.** ATP is known to participate in a number of different reflexes in the gastrointestinal tract (for reviews see Refs. 1 and 10) and, importantly, appears to act as a sensory mediator in the mucosa, generating action potentials in myenteric intrinsic sensory neurons via P2X receptors in guinea pig small intestine (1, 3). Gwynne and Bornstein (22) described a partial influence of ATP in amino acid-evoked local reflexes via P2X receptors, and in this study we investigated whether these receptors mediate fatty acid-evoked segmentation. Our results suggest that the action of ATP via P2X receptors is not critical to decanoic acid-induced segmentation, as luminal or bath application of PPADS had no effect on contractile activity. This suggests that fatty acid- and amino acid-evoked reflexes are distinct from one another and activated by separate
pathways involving specific neural mediators to modulate motor activity.

Our experimental data, together with modeling the effects of 5-HT and CCK on contractile activity, provide an explanation for the motor pattern evoked by luminal infusion of decanoic acid.

**Conclusions.** This study demonstrates that 5-HT plays an important role in the activation of neural circuits controlling nutrient-induced segmentation via a mechanism involving 5-HT$_3$ and 5-HT$_4$ receptors. We have also demonstrated a role for CCK in this activation via CCK-1 and CCK-2 receptors. Further investigation is required to determine the identity of other mediators of this reflex and to determine the location of receptors involved in segmentation evoked by other nutrients.

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**DISCLOSURES**

No conflicts of interest, financial or otherwise, are declared by the authors.

**AUTHOR CONTRIBUTIONS**

M.E. and J.D.C. performed the experiments; M.E. and J.D.C. analyzed the data; M.E., J.D.C., R.M.G., and J.C.B. drafted the manuscript; M.E., J.D.C., R.M.G., and J.C.B. edited and revised the manuscript; M.E., J.D.C., R.M.G., and J.C.B. approved the final version of the manuscript; R.M.G. and J.C.B. are responsible for conception and design of the research.

**REFERENCES**


