Enteric descending and afferent neural signaling stimulated by giant migrating contractions: essential contributing factors to visceral pain

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Sarna SK. Enteric descending and afferent neural signaling stimulated by giant migrating contractions: essential contributing factors to visceral pain. Am J Physiol Gastrointest Liver Physiol 292: G572–G581, 2007; First published September 21, 2006; doi:10.1152/ajpgi.00332.2006.—We investigated whether strong compression of an intestinal segment by giant migrating contractions (GMCs) initiates pseudoaffective signals from the gut, similar to those initiated by its distension with a balloon. The experiments were performed on conscious dogs by using close intra-arterial infusions of test substances that affect the receptors only in the infused segment. The stimulation of GMCs by close intra-arterial infusion of CGRP or distension of an intestinal segment by balloon increased the heart rate; the increase in heart rate was greater when the balloon distension and GMCs occurred concurrently in separate intestinal segments. The suppression of contractility in the distended segment blocked the increase in heart rate. By contrast, the stimulation of rhythmic phasic contractions (RPCs) or their spontaneous occurrence did not increase in heart rate. The occurrence of GMCs as well as intestinal distension also produced descending inhibition. The descending inhibition was blocked by the inhibition of nitric oxide synthase, but it was unaffected by the inhibition of adenyl cyclase, purinergic receptors P2X and P2Y, and muscarinic receptors M1 and M2. The synaptic transmission for descending inhibition was mediated primarily by nicotinic receptors and activation of nitric oxide synthase. It was unaffected by the inhibition of tachykinin receptors NK1, NK2, and NK3; serotonin receptors 5-HT1A, 5-HT2/5-HT1C, 5-HT3, and 5-HT4; and muscarinic receptors M1 and M2. The synaptic transmission for descending inhibition was mediated primarily by nicotinic receptors and activation of nitric oxide synthase. It was unaffected by the inhibition of tachykinin receptors NK1, NK2, and NK3; serotonin receptors 5-HT1A, 5-HT2/5-HT1C, 5-HT3, and 5-HT4; and muscarinic receptors. Our findings show that GMCs, but not RPCs, initiate pseudoaffective signals from the gut. In the presence of visceral hypersensitivity or impaired descending inhibition, the GMCs may become a noxious stimulus.

irritable bowel syndrome; inflammatory bowel disease; peristaltic reflex; nitric oxide; vasoactive intestinal peptide; high-amplitude propagating contractions; constipation; functional bowel disorders

INTERMITTENT ABDOMINAL CRAMPING of gut origin is one of the major symptoms in patients with irritable bowel syndrome (IBS) (13, 14) and inflammatory bowel disease (IBD) (2, 39). Visceral hypersensitivity has been identified as a contributing factor to this symptom (22, 28, 50, 67). However, the sensation of cramping in these patients is intermittent. Therefore, stable visceral hypersensitivity alone could not explain the intermittent occurrence of abdominal cramping. The sensation of cramping in these patients and in normal subjects is mimicked by intraluminal balloon distention beyond nociceptive threshold. This suggests that a mechanical stimulus from the gut wall is an essential requirement for the perception of intermittent pain of gut origin. This stimulus is likely to be the contractions of the gut wall. However, there is little information on which type or types of contractions (29, 57) may stimulate afferent signaling that is perceived to be painful by the central nervous system (CNS) or trigger pseudoaffective responses, such as increase in heart rate and abdominal wall contractions (52). The measurement of pseudo-affective reflexes is used routinely in animal studies as proxy for afferent signaling that may be perceived as painful in humans. Our first hypothesis is that giant migrating contractions (GMCs) (13, 14, 17, 21, 36, 43, 45, 54, 56) but not the rhythmic phasic contractions (RPCs) (57) stimulate afferent signaling that initiates pseudoaffective responses.

GMCs of the small intestine are large-amplitude (2–3 times greater than the maximum amplitude of RPCs during phase III activity of the migrating motor complex) and long-duration (4–6 times longer than the duration of an RPC) contractions (56). As a result, these contractions strongly occlude a 20- to 30-cm-long segment of the small intestine. Furthermore, these contractions propagate rapidly and uninterruptedly over long distances. The luminal contents trapped ahead of a GMC are, therefore, propelled rapidly over long distances (mass movements) (17, 45, 63). The large volume of luminal contents propelled rapidly by a GMC may distend the distal receiving segment. A GMC, therefore, may stimulate afferent signaling directly by strong compression of the intestinal wall and indirectly by distention of the distal receiving segment. The distention of the distal segment can be mimicked by inflation of an intraluminal balloon. Our second hypothesis is that the GMCs, but not RPCs, initiate the descending inhibition that allows the distal receiving segment to distend without stimulating afferent signaling and hence nociceptive perception. The impairment of the descending inhibition, however, may initiate afferent signaling concurrent with that produced by the GMCs so that the two afferent signals add up to exceed the nociceptive threshold, even in the absence of visceral hypersensitivity.

The descending inhibition in the gut is comprised of two components: 1) synaptic transmission via interneurons so that the inhibition can extend over a sizable length of the gut ahead of a GMC and 2) radial or lateral input to circular smooth muscle cells by enteric inhibitory motor neurons so that it can overcome the existing excitatory effects of the cholinergic excitatory motor neurons. Extensive in vitro studies have identified the neurotransmitters and receptor subtypes that mediate these two components of descending inhibition (9, 19, 32, 33, 42, 46). However, this information is not available in the intact conscious state. We, therefore, investigated the roles...
of key neurotransmitters and receptor subtypes involved in synaptic transmission in the interneurons and inhibition of smooth muscle contractions by the inhibitory motor neurons to produce descending inhibition in intact awake dogs. The antagonists were infused into the intestinal wall by close intra-arterial infusions in doses that are established to be selective in blocking their enteric targets but have no systemic effects (30, 40, 55, 58, 59, 68).

**EXPERIMENTAL METHODS**

**Surgical procedure.** The experiments were performed on 10 healthy conscious dogs of either sex. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the Zablocki Veterans Affairs Medical Center, Milwaukee, WI. Access to the abdominal cavity was obtained under general pentobarbital sodium anesthesia (30 mg/kg iv; Abbott Laboratories) to surgically attach strain-gauge transducers to the seromuscular layer to record circular muscle contractions and implant Silastic catheters in the ileal mesenteric arteries to infuse test substances directly into short segments of the ileum.

Three main adjacent mesenteric arteries labeled as proximal, middle, and distal, ~30 to 40 cm apart, were identified in the ileum. The arteries were freed carefully from the mesentery, preserving the nerves. A Silastic catheter (0.75 mm internal diameter, 1.63 mm external diameter) was inserted in the centripetal direction in a branch artery of each of the three main arteries so that its tip rested 1 to 2 mm from the junction of the branch artery and the main artery, as described previously (30, 40, 58). The infusion of saline at 15 to 20 ml/min for 10 to 15 s identified the boundaries of the infused segment. The segment refilled with blood within 2 to 3 s after the end of infusion. The infusion of saline at 1 ml/min for up to 10 min produced no apparent change in color of the segment and it did not stimulate any contractions. The length of the infused segment was limited to ~6 cm by ligating some secondary branch arteries, if necessary. Ties to the branch artery and the mesentery secured the catheters. Two or three strain-gauge transducers were attached to the seromuscular layer in each of the proximal (catheter 1), middle (catheter 2), and distal (catheter 3) infused segments to record circular muscle contractions.

A stainless steel cannula was implanted ~10 to 15 cm proximal to the site of the proximal catheter and one the same distance distal to the site of the distal catheter. A 6-cm-long balloon connected to a catheter was advanced through the proximal cannula to determine the position that placed the balloon at the site of the proximal infused segment. This mark was used later in experiments to position the balloon at this location to distend the proximal segment. The distal balloon was used for concurrent distension of its segment and stimulation of GMCs in the proximal segment. These two sites were far apart to interfere with each other.

The intraluminal and intra-arterial catheters were exteriorized subcutaneously in the subcapsular region. The catheters were housed in jackets that the dogs wore at all times. Each intra-arterial catheter was flushed twice daily with 2,000 IU of heparin. The dogs were allowed 5–7 days to recover from surgery.

**Experimental protocol.** All experiments were performed in the conscious state after an overnight fast. At least one phase III activity was recorded to establish the fasting state. The contractile signals were recorded on a 12-channel pen recorder (model 7D; Grass Instruments, Quincy, MA), with lower and upper cutoff frequencies set at direct current and 15 Hz, respectively.

All test substances were infused through the catheters at 1 ml/min during phase I or a quiescent period during phase II activity of the migrating motor complex. Close intra-arterial infusion of calcitonin gene-related peptide (CGRP) through the proximal catheter was used to stimulate GMCs, as described previously (58). The balloon was used to distend an intestinal segment, while measuring the pressure in the balloon with a gauge. Close intra-arterial infusions of methacholine (MCh) through the distal catheter were used to stimulate a series of RPCs. The antagonists of specific receptors were infused through the middle and distal catheters as described in RESULTS.

A waiting period of at least 30 min was allowed between successive infusions of agonists and antagonists. Preliminary experiments indicated that the responses to repeated infusions of agonists after this rest period were not different.

**Data analysis.** GMCs defined as contractions of duration four to six times longer and amplitudes two to three times larger than those of contractions in phase III activity of the migrating motor complexes were identified visually (56). The RPCs were quantified as area under contractions (WINDAQ/EX program; DATAQ Instruments, Akron, OH). The area under contractions was measured from the beginning of the first contraction after the start of infusion to the point at which the tracing returned to baseline and contractions ceased to occur.

All data are expressed as means ± SE. The n value represents the number of dogs. Statistical analysis was performed by analysis of variance with repeated measures. Student-Newman-Keuls test was used for multiple comparisons when the data were distributed normally, whereas Mann-Whitney’s rank sum test was used when the normality test failed; P < 0.05 was considered statistically significant.

**RESULTS**

**Pseudoaffective responses to balloon distension and GMCs.** We used the increase in heart rate to monitor the pseudoaffective response to distension of an intestinal segment with a balloon and its strong compression by a GMC. The distention of the proximal segment with a balloon for 5 min increased the heart rate in proportion to the distention pressure (Figs. 1A and 2A). The maximal increase in heart rate was achieved within the first minute of balloon distention and it was sustained during the entire 5-min distention period. The close intra-arterial infusions of 2 μM/min × 1 min CGRP stimulated one to four GMCs, some of which propagated distally. This dose of CGRP was shown earlier to consistently stimulate GMCs in the canine small intestine (58). The maximal increase in heart rate measured over 1-min during the occurrence of GMCs (135 ± 3%) was not significantly different from that attained during balloon distention to 290 mmHg (140 ± 6%) (Fig. 2B). However, the maximum increase in heart rate produced by the GMCs occurred only after one or two GMCs had begun to propagate. The maximal increase in heart rate when an intestinal segment distal to catheter 3 was distended to 290 mmHg concurrently with the stimulation of GMCs by CGRP in the
proximal infused segment (159 ± 4%) was significantly greater than that produced by each stimulus separately (Fig. 2B). The infusion of 1 ml/min × 5 min 0.9% saline had no significant effect on heart rate (100 ± 0 before vs. 92 ± 4 during saline infusion, n = 5).

We then investigated whether the increase in heart rate was due to strong compression of the intestinal segment by the GMCs or to the direct action of CGRP on its enteric neural receptors. The stimulation of GMCs by CGRP infusion was blocked either by a 1-min prior close intra-arterial infusion of 30 µM/min 1 min atropine or by starting the CGRP infusion during the third minute of infusion of 800 µM/min × 5 min infusion of verapamil, an L-type Ca2+ channel blocker. Both antagonists blocked the stimulation of GMCs by CGRP, as reported previously (58), and they also blocked the increase in heart rate (Figs. 1D and 3). Both agents also blocked the increase in heart rate produced by distention of the proximal segment with the balloon (Figs. 1C and 3). The balloon was distended 2 min after the start of infusion of verapamil. These data suggested that the suppression of contractility in an intestinal segment prevents afferent signaling from it in response to its distension or strong compression. Close intra-arterial infusions of atropine and verapamil alone had no significant effect on heart rate (100 ± 0 vs. 97 ± 4 after atropine infusion, n = 7 and 4, respectively). The control heart rates were measured within 5 min prior to the stimulus.

By contrast, close intra-arterial infusions of 2 µM/min × 5 min MCh stimulated a series of RPCs that had no significant effect on the heart rate (100 ± 0 vs. 95 ± 10 after 1-min infusion of MCh n = 5). Similarly, the heart rate did not vary with different intensities and frequencies of rhythmic phasic contractions during phases I, II, and III of the migrating motor complex (MMC) cycle in the ileum (100 ± 0, 96 ± 6 and 94 ± 5 in phases I, II and III, respectively, n = 5).

Neurotransmitters and receptors that mediate descending inhibition by balloon distention and GMCs. The following experiments investigated 1) the roles of putative neurotransmitters and receptors associated with the inhibitory motor neurons and smooth muscle cells that mediate descending inhibition and 2) the roles of selective neurotransmitters and receptors that mediate synaptic transmission during descending inhibition. They also investigated whether balloon distention that is normally used as an experimental stimulus and GMCs that occur spontaneously in the intact conscious state (45, 56) use the same or different neural signaling pathways to produce descending inhibition.

Balloon distention to 290 mmHg or stimulation of GMCs by close intra-arterial infusion of CGRP in the proximal segment was employed as stimulus to produce descending inhibition. The descending inhibition by balloon distention was measured by determining its effect on a series of RPCs stimulated at the site of the distal catheter by close intra-arterial infusion of 2 µM/MCh for 1 min (Fig. 4A). MCh was infused during the third minute of balloon distention (Fig. 4B). Initial experiments
indicated that descending inhibition produced by balloon distention was almost immediate and it was sustained during the entire period of distention. The area under RPCs stimulated by MCh without balloon distention was taken as 100%.

For GMC experiments, the RPCs were stimulated by a 5-min infusion of MCh at the site of the distal catheter (Fig. 5A). The descending inhibition produced by GMCs was not immediate upon their onset. The GMCs propagated distally at a velocity of 0.29 ± 0.02 cm/s (Fig. 5B). The first GMC had to propagate to a distance of 37 ± 4 cm before the MCh-induced contractions began to be inhibited. At this time, the GMCs were ~30 cm from the site of the distal catheter. The CGRP infusion at the proximal catheter and MCh infusion at the distal catheter were begun at the same time (Fig. 5C). The area under the contractions during the fifth minute of infusion of MCh and without the concurrent infusion of CGRP was taken as 100%. The inhibitory effect of propagating GMCs on MCh-induced RPCs always began before the start of the fifth minute of MCh infusion.

Balloon distention as well as stimulation of GMCs at the site of the proximal catheter significantly inhibited the contractions stimulated by MCh at the site of the distal catheter (Figs. 4B, 5B, and 6, respectively). The inhibition of nitric oxide (NO) synthase (NOS) at the site of catheter 3 by 10 mM/min × 1 min infusion of N-nitro-l-arginine methyl ester (l-NAME) given 12-min prior to the infusion of MCh blocked the balloon and GMC-induced descending inhibitions (Figs. 4C, 5D, and 6). However, the inhibition of adenyl cyclase by infusion of 1 μM/min × 5 min MDL-1233A; M1 receptors by infusion of 2 μM/min × 5 min pirenzepine; M2 receptors by 2 μM/min × 5 min infusion of methoctramine; P2X receptors by 2 μM/min × 5 min infusion of PPADS; P2Y receptors by infusion of 200 μM/min × 5 min infusion of suramin or 100 μM/min × 5 min infusion of reactive blue (data not shown) did not block descending inhibition by balloon or GMCs (Fig. 6). The depletion of norepinephrine by intravenous administration of 7.5 μM/min × 1 min of guanethidine also had no significant effect on descending inhibition produced by balloon distention or by GMCs. The effective concentrations of close intra-arterial infusions of antagonists were determined by their ability to block the inhibition of RPCs by their respective agonists (Fig. 7) or from the literature (30, 38, 40, 55, 59, 68).

The roles of specific neurotransmitters and their receptors involved in synaptic transmission for descending inhibition were determined by distending the balloon in the proximal segment, stimulating RPCs in the distal segment, and blocking selective receptors in the middle segment by close intra-arterial infusions. The inhibition of nicotinic receptors by infusion of 70 mM/min × 1 min hexamethonium or of NOS by 10 mM/min × 1 min infusion of l-NAME in the middle segment blocked the descending inhibition produced by balloon distention (Fig. 8A). On the other hand, the infusion of 30 μM/min × 1 min atropine or 1 μM/min × 1 min of 0.9% saline (not shown) in the middle segment had no effect on descending inhibition produced by balloon distention (Fig. 8A).

Furthermore, the blockade of NK1, NK2, and NK3 receptors by 1.6 μM/min × 5 min infusions of l-703.606, 1-659.877, and [Trp7, β, Ala8] neurokinin A4–10, respectively, and of 5-HT1A, 5-HT2/5-HT1C, 5-HT3/5-HT4 receptors by 2 μM/min × 5 min infusions of NAN-190 HBr2, LY-53857, tropesitron, and SDZ-205557, respectively also had no significant effect on descending inhibition produced by balloon distention (Fig. 8A).

DISCUSSION

Our findings show that the distention of an intestinal segment by an intraluminal balloon or its strong compression by GMCs stimulates centrally detectable afferent signals (pseu-
do-affective responses) that increase the heart rate. The increase in heart rate stimulated by intestinal distention or its strong compression by GMCs is of the same order of magnitude. However, the increase in heart rate by compression of an intestinal segment by GMCs and concurrent distension of another intestinal segment by balloon is greater than that due to each stimulus alone. In animal models of visceral pain, the initiation of pseudo-affective responses, such as abdominal wall contractions and increase in heart rate, have been used as markers of stimuli that can be perceived as painful (52). Our findings show, therefore, that the strong compression of a gut segment by a GMC can induce the sensation of pain. By contrast, the RPCs of maximal amplitude and frequency during phase III activity of the migrating motor complex may not be able to induce the sensation of pain.

Intermittent visceral pain of gut origin is one of the major symptoms of IBS (26, 45, 70, 67), IBD, and gut inflammation (2, 12, 25, 44, 47, 66, 67). The initial hypothesis was that this pain was due to motility dysfunction (60, 65), but there was little evidence to support this hypothesis. The occurrence of GMCs as distinct contractions was not widely known at that time, and the focus was on RPCs. More recently, the motility hypothesis has been discounted and the alternate hypothesis that the symptom of intermittent pain is almost entirely due to hypersensitivity of afferent sensory neurons and/or overinterpretation of these signals in the CNS has been advanced (10, 16, 22, 50). It has been proposed also that the motility dysfunction in IBS results from afferent neural hypersensitivity (10), but there is no evidence to support it. Although hypersensitivity of the afferent neurons is well established in a subset of IBS patients (1, 18, 70), it alone does not explain the intermittent occurrence of abdominal cramping. In a clinical laboratory setting, the patients complain of abdominal pain only when a balloon is distended in their gut lumen above the nociceptive threshold, even though hypersensitivity is continually present. This suggests that a mechanical event in the gut is required to stimulate afferent signals to perceive pain of gut origin. Our findings show that this pathophysiological mechanical event is the GMC. The afferent signaling stimulated by GMCs is similar to that stimulated by balloon distention. The RPCs are almost always present somewhere in the gut. If these contractors were the stimulus for pain, the pain would be present at all times. Furthermore, our findings show that RPCs do not generate centrally detectable afferent signals.

The GMCs occur spontaneously in normal healthy subjects two to six times per day (3, 6, 13, 43, 48, 51, 56, 57, 62). These spontaneous GMCs in health occur primarily in the terminal ileum and in the proximal colon; in the distal colon they also precede defecation (3, 37, 43, 48). In all cases, they produce mass movements (17, 45, 63), but they are seldom perceived to
be painful, even though they stimulate afferent signaling as shown by our findings. The reason seems to be that the afferent signals stimulated by the GMCs are still subthreshold for nociception, even though they exceed the affective threshold (Fig. 9). The afferent signaling by GMCs may be perceived as painful if their occurrence is accompanied with hypersensitivity of the afferent neurons and/or overinterpretation of the afferent signals in the CNS so that the nociception threshold is

Fig. 5. A: a 5-min infusion of MCh stimulated a series of RPCs at the site of the distal catheter. The area under these contractions at one of the strain-gauge transducers during the 5th minute of infusion was taken as 100% B: a close intra-arterial infusion of CGRP at the proximal catheter stimulated 2 GMCs that propagated distally. The MCh-stimulated RPCs at the site of the distal catheter were inhibited when the propagating GMCs reached ~30 cm from the site of RPCs. C: the inhibition of adenylyl cyclase by MDL-12330 A did not block descending inhibition ahead of distally propagating GMCs. D: the blockade of NOS by L-NAME almost completely blocked descending inhibition ahead of distally propagating GMCs.

Fig. 6. Bar graph showing the effects of L-NAME, MDL-12330 A (MDL), suramine, pyridoxal phosphate-6-azophenyl-2',4'-disulfonic (PPADS), pirenzepine (Piren.), methoctramine (Metho.), and guanethidine (Guan.) on descending inhibition ahead of a distally propagating GMC (A) and that produced by balloon (Bal.) distension at a proximal site (B). Only the inhibition of NOS blocked the descending inhibition in both cases, n = 5 or 6.
decreased or 2) when they are accompanied with impaired descending inhibition so that the afferent signaling stimulated by distension of the distal segment produced by mass movement and that by compression of the proximal segment by the GMC add up to a level above the nociceptive threshold (Fig. 9). Our findings show that when the contractility of the segment distended by a balloon is inhibited by atropine or verapamil, it does not stimulate afferent signaling.

Fig. 7. Representative tracings that show the method to determine the effective doses of various antagonists. First, MCh was infused close intra-arterially as control to stimulate a series of RPCs. Then the inhibitory neurotransmitter was infused concurrently to show that it blocks the MCh-induced contractions. Finally, different concentrations of the receptor antagonists were infused at the same site. An effective concentration was that which reversed the inhibitory effect of the neurotransmitter. SIN-1, 1,3-morpholinosydnoeimine.

Fig. 8. Antagonists (Ant.) of muscarinic receptors, nicotinic receptors, NOS (A), NK1, NK2, NK3 (B), 5-HT1A, 5-HT2, 5-HT1C, and 5-HT3/5-HT4 (C) were infused at the site of the middle catheter to determine whether they block descending inhibition produced by balloon distension at the proximal site. MCh was infused at the site of the distal catheter as control response to evaluate distending inhibition. Only the inhibition of nicotinic receptors and NOS blocked the descending inhibition (n = 5 or 6). Hex., hexamethonium.
Some IBS patients do not demonstrate visceral hypersensitivity and yet they have the symptom of intermittent abdominal cramping (1). This can happen particularly if the internal anal sphincter or the ileocelecal sphincter fails to relax ahead of a GMC and, therefore, not let the luminal contents pass through. Consequently, the segments proximal to them distend to add to the afferent signaling stimulated by the GMC itself. Therefore, a unifying hypothesis to explain the etiology of intermittent abdominal cramping may be that the occurrence of a GMC is the initiating event for nociceptive signaling, but it needs to be accompanied by visceral hypersensitivity and/or impaired descending inhibition for the signals to be perceived as painful.

The above unifying hypothesis is supported by several studies that show a correlation between the occurrences of GMCs in the small intestine or the colon and the sensation of intermittent pain in patients with IBS, IBD, or idiopathic constipation (2, 5, 13, 14, 44). On the other hand, the occurrence of GMCs in normal healthy subjects, who do not have visceral hypersensitivity or impaired descending inhibition, is not associated with pain. In normal healthy subjects, the occurrence and propagation of GMCs in the distal colon produces descending inhibition of the anal sphincter (37). It is also a common experience that abdominal pain occurs when defecation is withheld in the face of strong urge. A rapidly propagating GMC in the distal colon causes the urge to defecate because it pushes the fecal contents in the distal colon against closed anal sphincters and, therefore, distends the anal canal (3, 24, 37). This pain is relieved upon defecation because the expulsion of feces eliminates the stimulus that initiates the GMCs. It is well established that the frequency of GMCs is increased in patients with diarrhea-predominant IBS and IBD (4, 13, 15, 41, 62), which is associated with an increase in the frequency of abdominal cramping.

Electrophysiological recordings from afferent neurons in anesthetized animals show that each contraction or distention in the gut stimulates afferent signaling whose intensity is proportional to the stimulus amplitude (8, 20, 61). It is likely that, because of their large amplitude and long duration, the GMCs trigger high-threshold sensory fibers, whereas the RPCs trigger low-threshold fibers (11). The central destinations of high- and low-threshold fibers may be different so that the activation of high-threshold fibers is perceived to be painful, whereas that of the low threshold fibers is not. The silent fibers that are activated in response to peripheral injury may also be triggered by large-amplitude GMCs to enhance afferent signaling in disease states (27).

The descending inhibition, a part of the peristaltic reflex, has been studied extensively in in vitro studies using flat sheet preparations and ex vivo segments (7, 34, 64). The relaxation of tone in a prestretched flat sheet or electrical recordings of inhibitory junction potentials were used as end points in response to muscle stretch, mucosal stroking, or balloon-induced distortion of flat sheets of gut segments in these studies. In our study, we used GMC, which occurs spontaneously but infrequently in the intact conscious state, as the stimulus. The descending inhibition in in vitro experiments is measured over 1- or 2-cm length. It is not known whether this inhibition extends over a sizable length of the gut, such as ~30 cm observed in the intact conscious state in our experiments.

The descending inhibition due to balloon distention was immediate, but that due to stimulation of a GMC was delayed until the GMC had spread over a sizable length of the intestine. The delay may be due to two reasons. First, that the GMC may take some time to spread over a sizeable length of intestine, which may be required to generate enough stimulus strength to induce descending inhibition. Our data with heart rate also showed that the GMC had to propagate within this distance of the distal segment to produce the inhibition of contractions in it.
Several neurotransmitters of the inhibitory motor neurons (e.g., NO, VIP and ATP) and of interneurons (tachykinins, serotonin, opioids, and other neuropeptides) and their receptors have been identified in the gut wall (26). A potential role of several of these mediators in descending inhibition has also been reported in vitro preparations (9, 31, 33, 35, 64). However, our findings in the intact conscious state show that the synaptic transmission for descending inhibition is mediated primarily by nicotinic receptors and generation of NO in the interneurons, whereas the descending inhibition of smooth muscle contractions is mediated primarily by NO release from the inhibitory motor neurons. NO synthase has been identified in the interneurons (26); however, the mechanism by which NO regulates synaptic transmission for descending inhibition remains to be identified.

VIP inhibits smooth muscle contractions by the activation of adenyl cyclase followed by generation of cAMP. We found that the inhibition of adenylate cyclase by MDL-122530A effectively blocked the inhibition of MCh-induced RPCs by exogenous VIP. However, this antagonist had only minor effect on balloon- or GCM-induced descending inhibition, whereas the inhibition of NOS with l-NNAME blocked it almost completely. It is likely that the minor effect of VIP may be through the generation on NO as has been reported previously (32). Other investigators also failed to find a prominent role of endogenous VIP in inhibition of smooth muscle contractions (69) and concluded that its inhibitory effect depends on the experimental method used (23). By contrast, endogenous NO inhibits smooth muscle contractions in almost all experimental preparations.

In vitro studies show that the descending inhibition by muscle stretch is mediated by extrinsic neurons involving the prevertebral ganglia, whereas that induced by mucosal stroking is mediated by the descending interneurons (34). Our findings show that in the intact conscious state the descending inhibition by balloon distension that is equivalent to muscle stretch in vitro is mediated entirely by descending intrinsic neurons: the blockade of enteric nicotinic receptors by hexamethonium totally blocked this inhibition. Surgical myotomy also blocks descending inhibition (53).

In conclusion, GMCs, but not RPCs, stimulate afferent and descending neural signaling that produces pseudo-affective responses and descending inhibition, respectively. Numerous studies have reported the sensation of abdominal cramping with the occurrence of GMCs in IBS and IBD patients. The afferent signaling stimulated by GMCs in normal health is above pseudo-affective threshold, but it is subthreshold for nociception. However, if the sensory neurons are sensitized, as in IBS and IBD patients, to lower the nociceptive threshold, the same afferent signaling stimulated by GMCs may be perceived as painful. Alternately, if descending inhibition is impaired in a motility disorder, the afferent signaling due to strong compression of the intestine and distention of the distal receiving segment in the absence of relaxation of its tone and inhibition of spontaneous contractions add up to exceed the nociceptive threshold. The occurrence of GMCs, therefore, may be the central event to precipitate the sensation of intermittent abdominal cramping. The synaptic transmission to produce descending inhibition is mediated primarily by nicotinic receptors and release of NO, and inhibition of smooth muscle tone and spontaneous contractions is mediated by release of NO at the neuromuscular junction.

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