The Journal of Neuroscience, May 1994, 14(5): 2854–2860

Distinct Populations of Sensory Neurons Mediate the Peristaltic Reflex Elicited by Muscle Stretch and Mucosal Stimulation

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Recent studies suggest that muscle stretch and mucosal stimulation elicit intestinal peristalsis by activating distinct populations of sensory neurons that converge on the same population of enteric motor neurons. The present study sought to characterize the origin and projections of these sensory neurons. The reflex was elicited by applying muscle stretch and mucosal stroking to the central compartment of a three-compartment flat-sheet preparation of rat colon while ascending contraction and descending relaxation were measured in the orad and caudad compartments, respectively. Identical graded responses were elicited by muscle stretch and mucosal stimulation: atropine (1 μM) and the tachykinin antagonist spantide (10 μM) inhibited ascending contraction when added to the orad compartment only, while the vasoactive intestinal peptide antagonist VIP10-28 (10 μM) and the NO synthase inhibitor N^G-nitro-L-arginine (100 μM) inhibited descending relaxation when added to the caudad compartment only. Addition of capsaicin (1 μM) to the central compartment for 30 min abolished ascending contraction and descending relaxation elicited by muscle stretch and mucosal stimulation. Recovery of response was complete when capsaicin was applied to the mucosa of the colon *in situ* and measurements made 1 d after, implying that at this low concentration capsaicin depleted sensory nerve terminals of their transmitter content. Each of the following procedures, (1) excision of the inferior mesenteric ganglion, (2) application of 30 mM capsaicin to the ganglion for 30 min, (3) application of capsaicin followed by excision of the mesenteric ganglion, and (4) severing the fibers between the inferior mesenteric and dorsal root ganglia, all performed 2–3 weeks previously, abolished ascending contraction and descending relaxation elicited by muscle stretch but not by mucosal stimulation. Identical results were obtained in separate experiments in which muscle stretch was applied to intact whole colonic segments. Finally, removal of the mucosa had no effect on the responses to muscle stretch. The results demonstrate that sensory neurons activated by mucosal stimulation are wholly intrinsic, whereas sensory neurons activated by muscle stretch are extrinsic with cell bodies in the dorsal root ganglia and axonal projections to the motor limb of the reflex.

[Key words: intestinal peristalsis, enteric nervous system, sensory neurons, intestine, smooth muscle, peripheral reflexes, autonomic nervous system]

The efferent motor limb of the intestinal peristaltic reflex evoked by stretch applied to circular muscle consists of two components: an orad or ascending contraction accompanied by excitatory junction potentials (EJPs) and a caudad or descending relaxation accompanied by inhibitory junction potentials (IJPs) (Costa and Furness, 1976; Grider and Makhlouf, 1986; Smith et al., 1990, 1992a,b; Yuan et al., 1991). The components reflect release of the excitatory motor transmitters acetylcholine (ACh) and the tachykinins substance P (SP) and neurokinin A (Grider and Makhlouf, 1986; Smith and Furness, 1988; Grider, 1989a; Smith et al., 1990), and the inhibitory motor transmitters vasoactive intestinal peptide (VIP) and nitric oxide (NO) (Grider and Makhlouf, 1986; Grider, 1993). Measurements of EJPs and IJPs (Smith and Furness, 1988; Smith et al., 1991, 1992a; Yuan et al., 1991) suggest that the reflex evoked by mucosal stimulation and muscle stretch is mediated by the same population of enteric motor neurons. Modulatory cholinergic (Grider and Makhlouf, 1986; Smith and Furness, 1988; Tonini and Costa, 1990) and noncholinergic (Grider and Makhlouf, 1987, 1992; Grider et al., 1987) interneurons link the enteric motor neurons to afferent sensory neurons.

The sensory neurons activated by muscle stretch and mucosal stimulation are distinct, converging on the same motor neurons (Bornstein et al., 1991a; Smith et al., 1992a). The two populations of sensory neurons can be activated concurrently by muscle stretch and mucosal stimulation leading to augmentation of the efferent response (Smith et al., 1991; Yuan et al., 1991). When only one stimulus is applied repeatedly the response to it declines without affecting the response elicited by the other (Smith et al., 1990, 1991, 1992a; Yuan et al., 1992). Recent studies using a flat-sheet preparation to measure separately orad and caudad mechanical and electrical responses show that the reflex evoked by muscle stretch is not affected by removal of the mucosa, implying that mucosal stimulation and muscle stretch activate distinct populations of sensory neurons (Ginzel, 1959; Costa and Furness, 1976; Yokoyama and Ozaki, 1980; Grider, 1989b; Smith et al., 1990).

It is not known whether the sensory neurons mediating the peristaltic reflex are wholly intrinsic or are extrinsic with axonal projections to the motor limb of the reflex. The fact that the reflex is retained in acutely isolated intestinal segments is consistent with the operation of intrinsic sensory neurons or extrinsic sensory neurons with intrinsic collateral projections to the motor limb of the reflex that survive acute extrinsic dener-

Received June 21, 1993; revised Oct. 14, 1993; accepted Oct. 26, 1993.

This work was supported by Grant DK34153 from the National Institute of Diabetes, and Digestive and Kidney Diseases.

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vation. These projections eventually degenerate as they would 1 or 2 weeks after surgical or chemical excision of an extrinsic sensory cell body or after severing the connections to its intrinsic sensory projections.

These possibilities were examined in the present study using a three-compartment flat-sheet preparation of rat colon. The peristaltic reflex was elicited by muscle stretch or by mucosal stimulation applied to the middle compartment while ascending contraction and descending relaxation were measured in the peripheral orad and caudad compartments. Measurements were done in different groups of rats 2 weeks after extrinsic surgical or chemical denervation using the sensory neurotoxin capsaicin (Holzer, 1991). The results indicate that the peristaltic reflex induced by muscle stretch is mediated by extrinsic sensory neurons with intrinsic axonal projections, whereas the peristaltic reflex induced by mucosal stimulation is mediated by intrinsic neurons. Both populations of sensory neurons converge on the same population of effector motor neurons.

Materials and Methods

Extrinsic surgical and chemical denervation of colonic segments. Male Sprague–Dawley rats (200–400 gm) were fasted overnight prior to surgery but were allowed free access to water. The rats were anesthetized with sodium pentobarbital (50 mg/kg, i.p.). The inferior mesenteric ganglion was exposed and the animal prepared in one of five ways. In group 1, the inferior mesenteric ganglion was carefully removed under a dissecting microscope (surgical ganglionectomy). In group 2, the ganglion was encircled with a cotton dam soaked in mineral oil to prevent the diffusion of capsaicin into adjacent tissue. The ganglion was then covered with a cotton pledget soaked in capsaicin (1% or 30 mM) and then rinsed with saline and dried with sterile cotton swabs (chemical ganglionectomy). This highly lipophilic sensory neurotoxin was dissolved in a vehicle composed of 10% Tween 80, 10% ethanol, and 80% saline. The treatment has been shown to destroy small unmyelinated C-fibers (Holzer, 1991). In group 3, the ganglion was treated with 30 mM capsaicin as described above and then removed surgically. In group 4, the nerve fibers connecting the ganglion to the spinal cord were severed while the ganglion was left intact. The fifth group was composed of animals treated in the same fashion as group 2 but without capsaicin (sham-treated animals). The animals were allowed to recover for 2–3 weeks, at which time a colonic segment was removed for measurement of the peristaltic reflex. The responses obtained in animals from the five groups were compared with responses obtained in control animals that did not undergo any surgical procedure but were maintained in the same animal facility for 2–3 weeks.

Effects of mucosal application of capsaicin. The sensory toxin capsaicin was applied directly to the mucosa in two groups of animals. In one group, a solution containing capsaicin (1 μM) was applied to the mucosa for 30 min; capsaicin was then washed away and the peristaltic reflex examined in the segment. In a separate group, an 8 cm segment of colon was temporarily sealed with ligatures at both ends and filled with 5 ml of a solution containing capsaicin (1 μM). After 30 min, the segment was rinsed three times with Krebs medium and the animals were allowed to recover overnight. The segments were removed the next day and the peristaltic reflex examined.

Measurement of the peristaltic reflex. Intact segments of rat colon were prepared for measurement of the ascending contraction and descending relaxation components of the peristaltic reflex as described in detail previously (Grider and Makhlouf, 1986). The midcolon was removed and anchored in a chamber containing Krebs–bicarbonate medium maintained at 37°C and gassed with 95% O_2, 5% CO_2. The composition of the Krebs–bicarbonate medium was (in mM) NaCl, 118; KCl, 4.8; KH_2PO_4, 1.2; $CaCl_2$, 2.5; $MgSO_4$, 1.2; NaH_2CO_3, 25; glucose, 11; and bovine serum albumin, 0.1%. Muscle stretch (range, 2–10 gm) was applied to the center of the segment using weights attached to a hook-and-pulley assembly: descending relaxation was measured caudad and ascending contraction measured orad to the site of stretch using force-displacement transducers attached to the circular muscle.

In separate studies, the colonic segments were opened and pinned flat with the mucosal side up in an organ bath that was divided into three compartments, each 2 cm^2 containing 2 ml of medium. The portions of the segment in the peripheral (orad and caudad) compartments were attached to force-displacement transducers to record circular muscle activity. Stimuli were applied to the middle of the central ("sensory") compartment. Two types of stimuli were used to elicit the reflex: graded muscle stretch was applied as described above and mucosal stroking was applied with a fine brush as described by Smith and Furness (1988). The mucosal stroking was graded using repeated strokes (2–10) at a rate of one stroke per second.

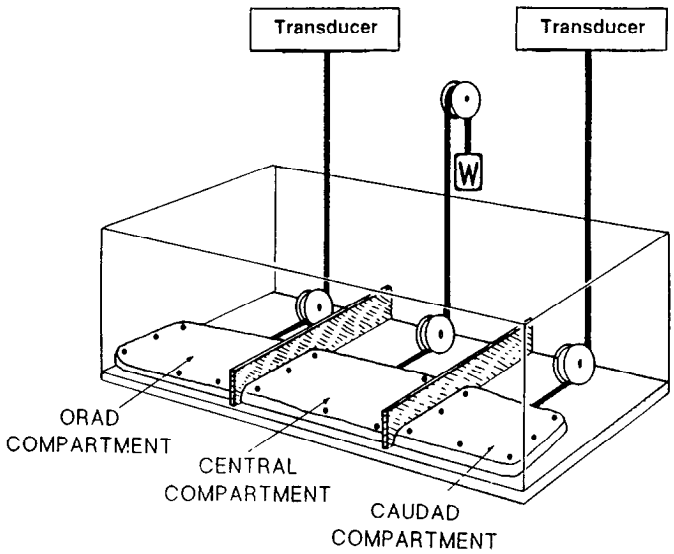

Figure 1. Compartmented flat-sheet preparation of rat colon for measurement of the peristaltic reflex. Ascending contraction was measured in the orad peripheral compartment and descending relaxation in the caudad peripheral compartment via force-displacement transducers attached to the muscle. Muscle stretch was applied to the central compartment by a hook-and-pulley assembly and mucosal stimuli were applied to the same compartment with a fine brush.

At the end of each experiment, the integrity of contractile and relaxant motor pathways was assessed by electrical field stimulation delivered at 80 V, 1 msec, and 5–16 Hz. The responsiveness of smooth muscle to contractile and relaxant agents was determined by addition of methacholine (0.1 mM) and vasoactive intestinal peptide (VIP; 1 μM), respectively. Relaxation induced by field stimulation or VIP was elicited in the presence of 1 μM atropine sulfate and 20 μM guanethidine sulfate and estimated from the decrease in resting muscle tension. Neither application of capsaicin to the inferior mesenteric ganglion nor ganglionectomy 2–3 weeks previously had any effect on the contractile and relaxant responses of the colonic segments to agonists or electrical field stimulation.

Data analysis. Descending relaxation and ascending contraction were measured in millinewtons (mN) of force and expressed as a percentage of the maximal response elicited by a 10 gm stretch stimulus or an eight stroke mucosal stimulus. The data are means ± SEM of *n* experiments, where *n* represents the number of animals studied. Statistical significance was tested by Student's *t* test for paired values.

Materials. Tween 80, capsaicin, N^G-nitro-L-arginine (L-NNA), and atropine were purchased from Sigma Chemical Co. (St. Louis, MO), and vasoactive intestinal peptide, the substance P (SP) antagonist spantide, and VIP10-28, from Bachem (Torrance, CA).

Results

The peristaltic reflex induced by stretch in a compartmented colonic preparation

As previously shown, the ascending contraction and descending relaxation components of the peristaltic reflex can be elicited by muscle stretch in isolated intact colonic segments as well as in flat-sheet preparations (Grider and Makhlouf, 1986; Grider, 1989b). In the present study, both components of the reflex were

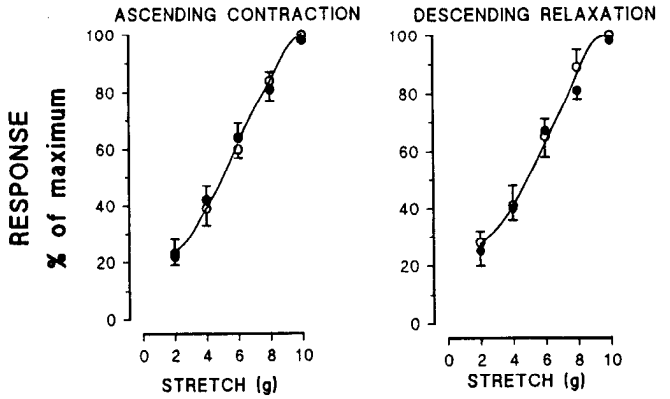

Figure 2. Ascending contraction orad and descending relaxation caudad to the site of muscle stretch. Identical responses were obtained from isolated intact whole segments (○) and compartmented flat-sheet colonic preparations (●). Results are expressed as a percentage of maximal responses (contraction, 8.5–8.8 mN; relaxation, 4.1–4.4 mN). Values are means ± SE of 6–10 experiments.

elicited by applying graded stretch to the central compartment of a three-compartment preparation and measuring ascending contraction in the peripheral compartment orad and descending relaxation in the peripheral compartment caudad (Fig. 1). The features and magnitude of the response elicited in this fashion were identical to those elicited in an isolated intact whole segment (Fig. 2). The maximal ascending contractile responses elicited by 10 gm stretch in whole colonic segments and flat-sheet preparations were 8.5 ± 0.6 mN (n = 10) and 8.8 ± 1.6 mN (n = 10), and the maximal descending relaxant responses elicited by the same stimulus were 4.1 ± 0.7 and 4.4 ± 0.6 mN.

Addition of atropine (1 μM) to the orad compartment abolished ascending contraction induced by low grades of stretch and partly inhibited contraction elicited by higher grades of stretch (Fig. 3). Addition of the SP antagonist spantide (10 μM) had no effect on contraction elicited by low grades of stretch and partly inhibited contraction elicited by higher grades of stretch (Fig. 3). A combination of both antagonists abolished ascending contraction elicited by all grades of stretch. Addition of atropine or spantide to the caudad compartment had no effect on ascending contraction.

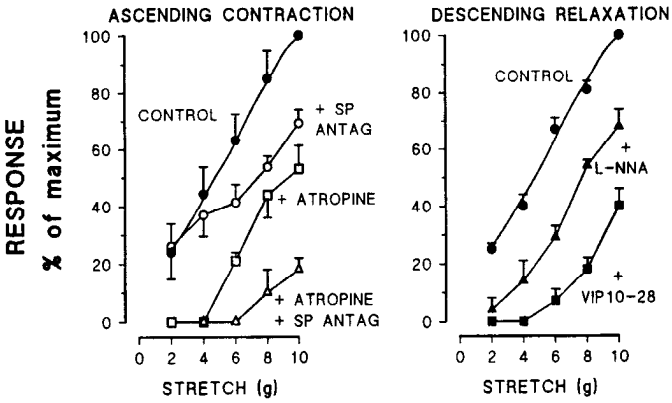

Figure 3. *Left,* Effect of atropine (1 μM), SP antagonist (10 μM), and a combination of both on ascending contraction. *Right,* Effect of VIP10-28 (10 μM) and the NO synthase inhibitor L-NNA (100 μM) on descending relaxation. The responses were elicited by muscle stretch applied to the central compartment of a flat-sheet colonic preparation. Results are expressed as a percentage of maximal response. Values are means ± SE of three or four experiments.

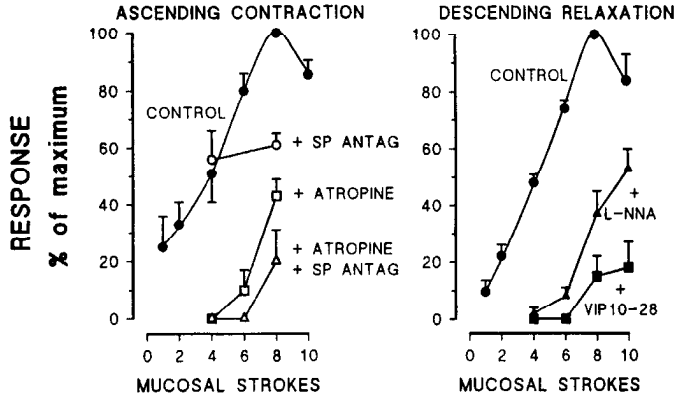

Figure 4. *Left,* Effect of atropine (1 μM), SP antagonist (10 μM), and a combination of both on ascending contraction. *Right,* Effect of VIP10-28 (10 μM) and the NO synthase inhibitor L-NNA (100 μM) on descending relaxation. The responses were elicited by mucosal strokes applied at the rate of one per sec (range 1–10 strokes) to the central compartment of a flat-sheet colonic preparation. Maximal responses were similar to those elicited by muscle stretch (contraction, 10.8 mN; relaxation, 4.0 mN). Results are expressed as a percentage of maximal response. Values are means ± SE of three to eight experiments.

Addition of the VIP antagonist VIP10-28 (10 μM) to the caudad compartment inhibited descending relaxation, abolishing the response elicited by low grades of stretch (Fig. 3). VIP10-28 was more effective than the NO synthase inhibitor L-NNA (100 μM) in inhibiting descending relaxation (Fig. 3), a feature also observed in intact colonic segments (Grider, 1993). The relative potency of VIP10-28 reflects the fact that VIP-induced NO production in muscle cells is the main source of NO (Grider et al., 1992; Grider, 1993; Murthy et al., 1993). Addition of VIP10-28 and L-NNA to the orad compartment had no effect on descending relaxation.

The peristaltic reflex induced by mucosal stimulation in a compartmented colonic preparation

Graded stroking of the mucosa in the central compartment elicited ascending contraction in the peripheral orad compartment and descending relaxation in the peripheral caudad compartment in proportion to the number of strokes applied (range of 1–10 strokes) (Fig. 4). The features and magnitude of the responses were similar to those elicited by muscle stretch applied to the central compartment (compare Figs. 2–4). Maximal ascending contraction (10.8 ± 1.0 mN; n = 13) and descending relaxation (4.0 ± 0.4 mN; n = 13) elicited by eight mucosal strokes were not significantly different from those elicited by 10 gm muscle stretch in the same preparations.

Addition of atropine (1 μM) to the orad compartment abolished ascending contraction induced by submaximal stimulation (<6 strokes) and partly inhibited contraction elicited by 8–10 strokes (Fig. 4). Addition of spantide (10 μM) had no effect on contraction elicited by submaximal stimuli and partly inhibited contraction elicited by maximal stimuli (Fig. 4). A combination of both antagonists was additive (80–100% inhibition). Addition of atropine or spantide to the caudad compartment had no effect on ascending contraction.

Addition of VIP10-28 (10 μM) to the caudad compartment inhibited descending relaxation abolishing the response elicited by submaximal stimuli (<6 strokes) (Fig. 4). VIP10-28 was more effective than the NO synthase inhibitor L-NNA (100 μM) in inhibiting descending relaxation (Fig. 4), a feature also ob-

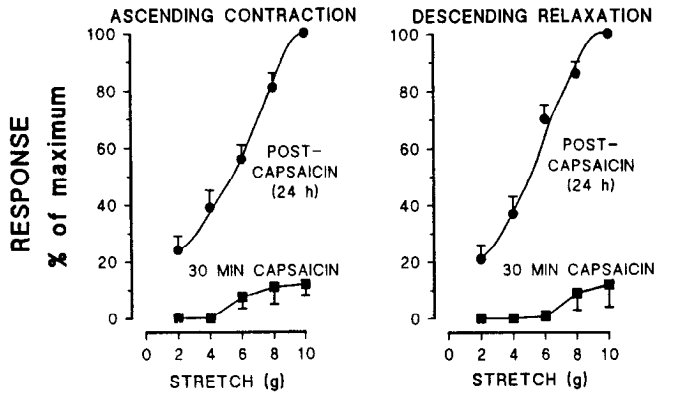

Figure 5. Effect of capsaicin (1 μM) on ascending contraction and descending relaxation elicited by muscle stretch applied to central compartment of a flat-sheet colonic preparation. ■, measurements made immediately after 30 min application of capsaicin to the mucosa in the central compartment; ●, measurements made 1 d after application of capsaicin for 30 min to the mucosa of the colon *in situ*. Results are expressed as a percentage of maximal responses. Values are means ± SE of four or five experiments.

served in the response to muscle stretch (compare Figs. 3, 4). Addition of VIP10-28 and L-NNA to the orad compartment had no effect on descending relaxation.

The results depicted in Figures 2–4 show that the same transmitters responsible for ascending contraction (ACh and tachykinins) and descending relaxation (VIP and NO) were released by muscle stretch or mucosal stimulation.

The mucosa was removed by blunt dissection in some rats to determine the effect on the stretch-induced peristaltic reflex. As shown previously in human intestine (Grider, 1989b), removal of the mucosa had no effect on ascending contraction or descending relaxation elicited by muscle stretch (data not shown).

Effect of capsaicin applied to the mucosa on the components of the peristaltic reflex

In order to examine the sensory limb of the reflex, capsaicin (1 μM) was applied for 30 min to the mucosal surface in different compartments. Application of capsaicin to the mucosa in the

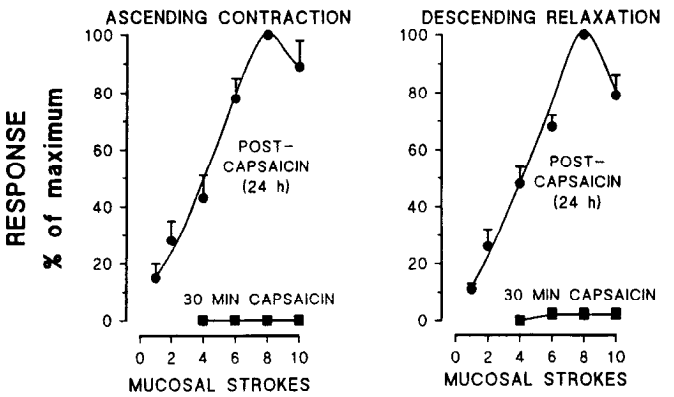

Figure 6. Effect of capsaicin (1 μM) on ascending contraction and descending relaxation elicited by mucosal stimulation applied to central compartment of a flat-sheet colonic preparation. ■, measurements made immediately after 30 min application of capsaicin to the mucosa in the central compartment; ●, measurements made 1 d after application of capsaicin for 30 min to the mucosa of the colon *in situ*. Results are expressed as a percentage of maximal responses. Values are means ± SE of four to six experiments.

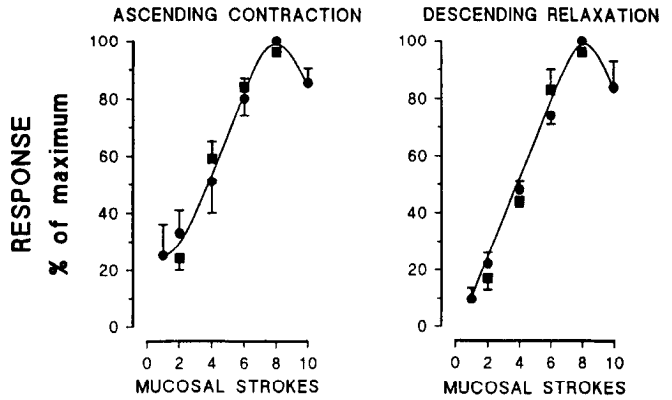

Figure 7. Ascending contraction and descending relaxation elicited by mucosal strokes applied to the central compartment of a flat-sheet colonic preparation. ●, control rats; ■, rats in which the inferior mesenteric ganglion was removed 2–3 weeks previously. Results are expressed as a percentage of maximal responses. Values are means ± SE of six experiments.

central compartment abolished ascending contraction and descending relaxation elicited by muscle stretch (Fig. 5) or mucosal stimulation (Fig. 6). Application of capsaicin to the mucosa in either the orad or the caudad compartment had no effect on ascending contraction or descending relaxation.

In separate experiments capsaicin was applied for 30 min to the mucosa of the colon *in situ* and the animals allowed to recover. Measurements of the reflex the next day showed that ascending contraction and descending relaxation in response to muscle stretch and mucosal stimulation had recovered completely (Figs. 5, 6).

Effect of extrinsic denervation on the peristaltic reflex elicited by mucosal stimulation and muscle stretch in flat-sheet preparations of colon

The effect of extrinsic denervation was determined in separate groups of rats in which the inferior mesenteric ganglion was resected and/or treated with 30 mM capsaicin 2–3 weeks previously. Neither ganglionectomy nor treatment with 30 mM capsaicin had any effect on ascending contraction or descending relaxation (Figs. 7, 8) elicited by mucosal stimulation applied to the central compartment of flat-sheet colonic preparations.

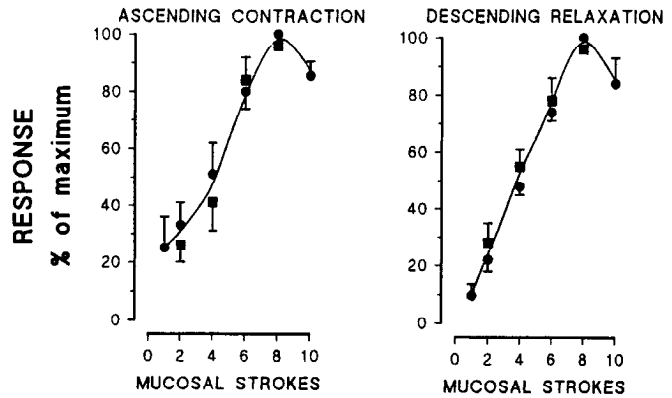

Figure 8. Ascending contraction and descending relaxation elicited by mucosal strokes applied to the central compartment of a flat-sheet colonic preparation. ●, control rats; ■, rats in which capsaicin (30 mM) was applied for 30 min to the inferior mesenteric ganglion. Results are expressed as a percentage of maximal responses. Values are means ± SE of six experiments.

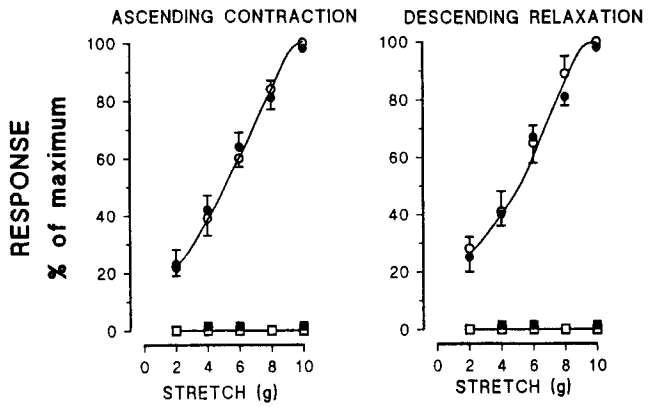

Figure 9. Ascending contraction and descending relaxation elicited by muscle stretch in flat-sheet preparations (●, ■) and whole segments (○, □) of rat colon. ●, ○, response in control rats; □, ■, response in rats in which the inferior mesenteric ganglion was removed 2–3 weeks previously. Results are expressed as a percentage of maximal responses. Values are means ± SE of four to six experiments.

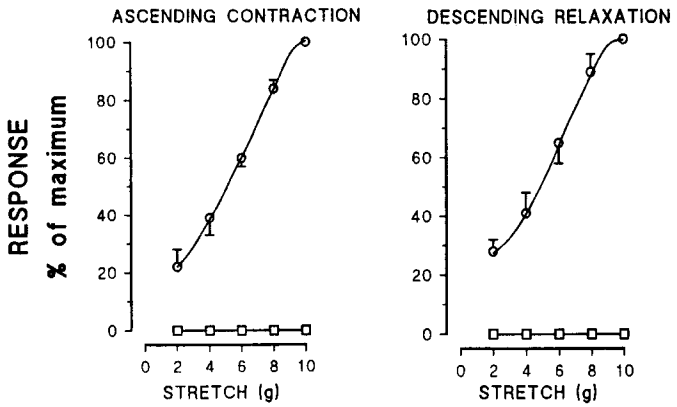

Figure 11. Ascending contraction and descending relaxation elicited by muscle stretch in intact whole segments of colon from control rats (○) and from rats in which the nerve fibers between the inferior mesenteric and dorsal root ganglia were severed 2–3 weeks previously (□). Results are expressed as a percentage of maximal responses. Values are means ± SE of three experiments.

Similar results were obtained in a group of animals treated with 30 mM capsaicin for 30 min followed by ganglionectomy (data not shown). The preservation of the response to mucosal stimulation 2–3 weeks after each treatment implied that the sensory neurons mediating the peristaltic reflex elicited by mucosal stimulation were wholly intrinsic.

In contrast, in the same groups, muscle stretch applied to the central compartment failed to elicit ascending contraction or descending relaxation whether or not it was preceded by mucosal stimulation. The results obtained with inferior mesenteric ganglionectomy and capsaicin treatment are depicted in Figures 9 and 10. Similar results were obtained when the two procedures were combined (data not shown).

The response to mucosal stimulation or muscle stretch in sham-operated animals was intact and the responses were similar to those depicted in Figures 2–4.

Effect of extrinsic denervation on the peristaltic reflex elicited by muscle stretch in isolated whole segments of colon

The effects of extrinsic denervation were also examined in separate groups of rats in which the inferior mesenteric ganglion was resected and/or treated with 30 mM capsaicin 2–3 weeks previously. In these groups, the measurements were done in isolated whole colonic segments as described previously. In rats subjected to ganglionectomy or treated with capsaicin, muscle stretch did not elicit ascending contraction or descending relaxation (Figs. 9, 10). Similar results were obtained in rats treated with capsaicin followed by ganglionectomy (data not shown). The results obtained in isolated whole segments were similar to those obtained in flat-sheet preparations.

In a separate group of rats in which nerve fibers between the inferior mesenteric and dorsal root ganglia were severed 2–3 weeks previously, muscle stretch did not elicit either component of the reflex (Fig. 11).

The absence of response to stretch in whole segments and flat-sheet preparations 2–3 weeks after extrinsic denervation implied that the cell bodies of sensory neurons mediating the response to muscle stretch are located in the dorsal root ganglion with fibers passing in close proximity to the inferior mesenteric ganglion.

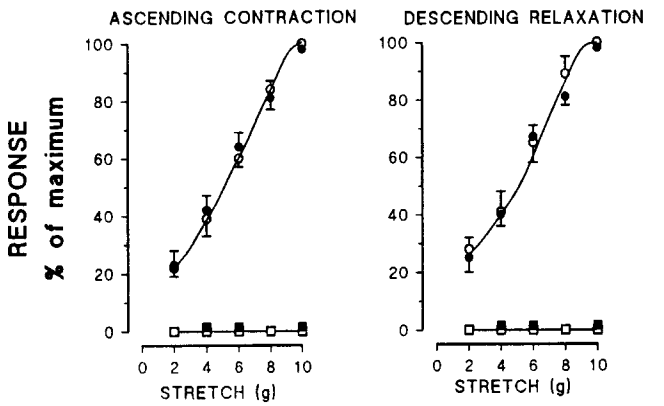

Figure 10. Ascending contraction and descending relaxation elicited by muscle stretch in flat-sheet preparations (●, ■) and whole segments (○, □) of rat colon. ●, ○, response in control rats; □, ■, response in rats in which the inferior mesenteric ganglion was treated for 30 min with 30 mM capsaicin 2–3 weeks previously. Results are expressed as a percentage of maximal responses. Values are means ± SE of four to six experiments.

Discussion

This study shows that distinct populations of sensory neurons are activated by muscle stretch and mucosal stimulation and converge on the same population of enteric motor neurons mediating the intestinal peristaltic reflex. The motor neurons responsible for ascending contraction release ACh and tachykinins (Grider and Makhlouf, 1986; Smith and Furness, 1988; Grider, 1989a,b; Smith et al., 1990), whereas the motor neurons responsible for descending relaxation release VIP and NO (Grider and Makhlouf, 1986; Grider, 1993). As shown elsewhere, NO is generated additionally by the action of VIP on target muscle cells (Grider et al., 1992; Grider 1993).

The sensory neurons activated by muscle stretch are extrinsic with cell bodies located in the dorsal root ganglion. Evidence in support of the existence of distinct populations of sensory neurons activated by stretch was obtained in the present study from experiments in which extrinsic nerve fibers innervating the colon were surgically or chemically destroyed by excision of the inferior mesenteric ganglion and/or application of a high concentration of capsaicin (30 mM). After allowing 2–3 weeks for degeneration of intrinsic projections, the peristaltic reflex could not be elicited by muscle stretch but was elicited by mu-

cosal stimulation. The peristaltic reflex could not be elicited by muscle stretch also when only the fibers connecting the inferior mesenteric and dorsal root ganglia were severed, thus establishing the location of the sensory cell bodies at a site proximal to the inferior mesenteric ganglion, probably in the dorsal root ganglion. The fact that the peristaltic reflex could be elicited after acute isolation of the colon suggests that the sensory neurons activated by muscle stretch possess axonal projections to enteric neurons within the wall of the intestine; these projections are retained for a period and can mediate the reflex but they eventually degenerate.

The sensory neurons activated by mucosal stimulation are wholly intrinsic with cell bodies located in the enteric nervous system. Neither chemical nor surgical extrinsic denervation affected the peristaltic reflex elicited by mucosal stimulation. It could not be ascertained whether the cell bodies of this population of neurons were located in the myenteric or submucosal plexus; their nerve endings, however, were located in the mucosa since excision of the mucosa abolished the response to mucosal stimulation.

The sensory terminals activated by mucosal stimulation are located in the mucosa whereas those activated by muscle stretch are located in deeper regions. This notion is supported by the fact that removal of the mucosa abolished the response to mucosal stimulation but had no effect on the response to muscle stretch (Ginzel, 1959; Costa and Furness, 1976; Grider, 1989b; Yuan et al., 1990). The terminals in both locations were susceptible to mucosal application of capsaicin (1 μM), which at this low concentration depleted the nerve terminals of their sensory transmitters (Holzer, 1988, 1991). The effect of capsaicin was transient since the reflex could be fully elicited the following day.

The existence of distinct populations of sensory neurons activated by muscle stretch and mucosal stimulation is supported by electrophysiological studies using EJPs and IJPs as indices of ascending contraction and descending relaxation, respectively (Bornstein et al., 1991a; Smith et al., 1992a). The neurons could be differentially desensitized by repeated stimulation and when activated concurrently could be shown to converge on the same population of enteric motor neurons (Smith et al., 1990, 1991, 1992a; Yuan et al., 1991, 1992). These studies, unlike the present one, did not identify the distinct locations of the sensory neurons.

The results of the present study are consistent with those obtained by Bulbring et al. (1958) in a Trendelenburg preparation where neither celiac nor dorsal root ganglionectomy had any effect on the reflex elicited by intraluminal distension, whereas removal of the mucosa abolished the reflex. The pattern of response implied that the reflex evoked by distension in this preparation was mediated by the mucosal terminals of intrinsic sensory neurons. Unlike intraluminal distension, muscle stretch applied directly to circular muscle as in the present study activates sensory pathways that are not affected by removal of the mucosa.

There is some evidence that the intrinsic sensory neurons may be AH/Dogiel type II neurons based on the their morphology, calbindin immunoreactivity, axonal projections, and paucity of synaptic input from other enteric neurons (Nishi and North, 1973; Iyer et al., 1988; Pompolo and Furness, 1988; Bornstein et al., 1991b). Kirchgessner et al. (1992) have recently shown that stimulation of guinea pig intestinal mucosa induces the expression of Fos in a subset of calbindin-immunoreactive neurons. The effect was blocked by 5-HT antagonists, suggesting activation of mucosal sensory endings by 5-HT released from mucosal stores. It is not known whether the neurons activated in this fashion subserve the peristaltic reflex or other reflexes, for example, secretory reflexes, elicited by mucosal stimulation. Earlier studies by Bulbring and Lin (1958), however, had shown that mucosal application of 5-HT to guinea pig and rabbit intestine can evoke propulsive activity.

The relative importance of muscle stretch and mucosal stimulation in initiating peristalsis depends on the region of the intestine. In the small intestine where the contents are fluid, mucosal stimulation is likely to be more effective, whereas in the colon where the contents are more solid, a combination of mucosal stimulation and muscle stretch should determine propulsion.

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