Synaptic transmission in simple motility reflex pathways excited by distension in guinea pig distal colon

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Bian, X.-C., L. F. Heffer, R. M. Gwynne, J. C. Bornstein, and P. P. Bertrand. Synaptic transmission in simple motility reflex pathways excited by distension in guinea pig distal colon. Am J Physiol Gastrointest Liver Physiol 287: G1017–G1027, 2004. First published July 15, 2004; doi:10.1152/ajpgi.00039.2004.—We examined specific receptor/transmitter combinations used at functionally identified synapses in ascending and descending reflex pathways of guinea pig distal colon. Excitatory (EJPs) or inhibitory junction potentials (IJPs) were recorded intracellularly from nicardipine-paralized circular smooth muscle in either the oral or anal recording chamber of a three-chambered organ bath, respectively. Blockade of synaptic transmission in the central chamber with a 0.25 mM Ca2+/12 mM Mg2+ solution abolished EJPs evoked by distension applied either in the central or the far (anal) chamber. IJPs evoked by distension in the central or the far (oral) chamber were depressed to ~50% of control. Hexamethonium (nicotinic receptor antagonist, 200 μM) in the central chamber reduced IJPs evoked by far or central distension to 50%, whereas EJPs evoked by far distension were abolished and EJPs evoked by central distension were reduced to 70% of control. Hexamethonium in the recording chambers reduced both IJPs and EJPs evoked by central distension to ~50%. EJPs in the ascending pathway were unaffected by blockade of muscarinic receptors in the central chamber or blockade of neurokinin 3 tachykinin receptors in this or the recording chamber. In the descending pathway, blockade of P2 receptors in the same chambers had only a minor effect on distension-evoked IJPs. Thus some intrinsic sensory neurons of guinea pig colon have long descending projections (>30 mm), but ascending projections of <15 mm. In contrast to the ileum, transmission between ascending or descending interneurons and from sensory neurons to descending interneurons is predominantly via nicotinic receptors; but transmission to inhibitory or excitatory motoneurons and from sensory neurons to ascending interneurons involves nicotinic and other unidentified receptors.

enteric nervous system; electrophysiology; smooth muscle; enteric reflex

THE COLON IS SPECIALIZED FOR the absorption of water and electrolytes and the storage and transport of material. The digestion and absorption of nutrients (such as those produced from bacterial fermentation) is of lesser overall importance. The intrinsic circuitry underlying colonic behavior may thus be quite different from that of the small intestine in which mixing and primary nutrient absorption are crucial functions. These differences are currently not well defined but are critical for interpretation of a vast range of currently available data.

The guinea pig small intestine has been an excellent model for studying the circuitry of the enteric nervous system. It is in this system that the best data on projection patterns, chemical code, and electrophysiology have been gathered and correlated to characterize specific functional classes of neuron (8) and to reveal the nature of synaptic transmission at specific classes of synapses (7). The circuitry of the ileum, in particular, provides a template on which to design studies investigating the circuitry in the colon.

Neuroanatomical studies (31, 33, 34) have revealed substantial similarities between the circuitry in the ileum and colon, but have also highlighted some significant differences. For example, one class of somatostatin-containing interneurons appears to have descending projections in the ileum but projects orally in colon (29). There is only a single class of ascending interneuron in the ileum, but there are four such classes in the distal colon. Interestingly, recent work in the colon has identified one class of neurons with the morphology of descending inhibitory interneurons, but which was also distension-sensitive (40–42). Finally, the electrophysiological characteristics of colonic neurons (10, 28, 30, 45) and the whole organ pharmacology (e.g., see Ref. 50) are subtly different from those of the ileum. For example, 5-HT or GABAA receptor antagonists inhibit colonic reflexes (e.g., see Refs. 21 and 25), but have little effect in the ileum (for review, see Ref. 6).

As might be expected, the peristaltic reflex differs between the ileum and the colon. For example, when the muscle is free to contract, a stationary distension of the gut wall causes a descending excitatory reflex in ileum (39) but an initial descending inhibitory reflex in the colon (12, 15, 38). In guinea pig ileum, there is very little cholinergic transmission at any of the functionally identified synapses within the descending inhibitory reflex pathway (4, 24) or in the descending excitatory reflex pathway (35). In rat and guinea pig colon, when nicotinic receptors were blocked at all synapses, ascending and descending reflexes were abolished (19); when each functional class of synapse in the descending inhibitory pathway of rat colon was examined in detail, these were all found to be nicotinic (5). Many other transmitters, such as GABA, somatostatin and vasoactive intestinal peptide, are important and are likely to have a role in the circuitry of the colon (e.g., see Refs. 20 and 25). However, it is our understanding of the fundamentals in this region that is lacking most. For example, the role of nicotinic transmission at identified synapses in the ascending excitatory or descending inhibitory reflexes is still unknown (for review, see Ref. 6), and little is known about the lengths of the functional neural projections underlying these reflexes.

In the present study, we analyzed the nature of transmission at specific classes of synapses within the ascending and descending reflex pathways of the guinea pig distal colon to
identify how such synapses may contribute to the overall behavior of this organ. The divided organ bath methodology, which allows functional classes of synapse to be isolated and studied pharmacologically, was identical to that which has already provided detailed information about transmission at analogous classes of synapses in guinea pig ileum (e.g., see Refs. 4, 22, 24, and 49) and in the descending inhibitory pathway of rat distal colon (5), thereby allowing direct comparisons between systems.

MATERIALS AND METHODS

Preparation

Guinea pigs of either sex (200 to 350 g) were killed by a blow to the head and severing of the carotid arteries, a procedure approved by the University of Melbourne Animal Experimental Ethics Committee in accordance with the guidelines of the National Health and Medical Research Council of Australia. A segment of colon (5–10 cm long, taken 5–10 cm from the rectum), was taken and placed in a physiological saline (composition in mM: 118 NaCl, 4.8 KCl, 1.0 NaH2PO4, 25 NaCO3, 1.2 MgSO4, 11.1 D-glucose, and 2.5 CaCl2) and bubbled with 95% O2-5% CO2 at room temperature. After the lumen was flushed with physiological saline solution, the segment was cut open along the mesenteric border. The full-thickness segment (~50 mm length and 15 mm diameter, with myenteric and submucous plexes intact) was opened and pinned flat with the mucosal surface uppermost in a three-chambered organ bath and slowly warmed to 35°C (for schematic see Ref. 4). Each chamber had an ~5–ml volume and was separately perfused at 5 ml/min. Drugs or solutions added to one chamber did not affect tissues located in the other chambers. This bath was used for experiments on ascending or descending reflexes; the tissue was simply pinned in the orientation required.

Rubber balloons (5 mm diameter, embedded in the base of the bath) were used to distend the intestinal wall in the central chamber 15 mm from the recording site, and in the far chamber 30 mm away (e.g., Fig. 1A, left). The volume of the distension stimulus was increased progressively by 0.05 ml until a maximal amplitude junction potential was initiated. The volume used to obtain this initial maximal response was then used for the rest of the stimuli for that tissue; thus all later stimuli were assumed to be maximal or nearly maximal. For the same distension, the amplitude of the junction potential was decreased with an increasing distance of the balloon from the recording site. In some experiments, only the central and recording chambers were used. Intervals of ≥2–3 min between distension stimuli were used to avoid rundown of responses (48).

The saline solution used in all chambers contained the L-type calcium channel blocker nicardipine (2.5 μM). This causes the smooth muscle to relax, thereby allowing stable intracellular recordings to be made and is also likely to block any descending excitatory reflexes (e.g., in ileum, see Ref. 43). Blockade of the excitatory reflex allowed the descending inhibitory reflex to be studied without interference.

In some experiments on the descending inhibitory pathway, notably those in which drugs were added to the recording chamber, electrical stimulation of neurons within this chamber was used to provide an extra control. A pair of silver wires (0.5 mm diameter, 2 cm length) was placed in the recording chamber above and below the preparation and either parallel or perpendicular to the circular muscle, ~4 mm oral to the recording site so that transmural electric field stimulation (EFS) could be applied to the pathways and directly to motor neurons (e.g., see Ref. 4). Junction potentials evoked in response to EFS could be graded in amplitude according to stimulus intensity. The stimulus intensity of EFS was chosen to evoke junction potentials with amplitudes matching those of physiologically evoked junction potentials in the same preparation.

Electrophysiology

Glass microelectrodes were filled with 2 M KCl (80- to 100-MΩ tip resistance) and mounted on a mechanical micromanipulator (Leitz; Wetzlar, Germany) to record intracellularly from the circular smooth muscle. We found that impaling circular muscle through either the mucosa or the serosa was difficult in tissue from some animals. Thus for some preparations, a patch of mucosa and submucosa at one end was dissected away to allow direct impalement of the circular muscle. Responses recorded under these conditions did not differ from those seen when the circular muscle was impaled from the serosal side through the longitudinal muscle as has been done in previous studies. The potential difference across the cell membrane was recorded onto a personal computer using a computerized polygraph system (Axotape 2.0.2, Axon Instruments, Foster City, CA) and then analyzed with Origin 6.0 (MicroCal, Northampton, MA).

In each experiment, tissue from one animal was used to derive one n value for purposes of statistical comparison. Within each experiment, a set of three control excitatory (EJPs) or inhibitory junction potentials (IJPs) was evoked by distension or EFS (5), and the amplitudes were averaged. Blockers or receptor antagonists were then added to the superfusing solution and allowed to equilibrate for 5 to 10 min in one or more of the chambers. During the next 30 min, a second set of junction potentials was evoked in the presence of this changed solution. Finally, these solutions were washed out for 30–60 min, and a third set of junction potentials was evoked.

Statistics

Unless noted otherwise, statistical analyses were performed by using Student’s paired t-tests to compare junction potential amplitudes (mean of 3 replicates) before and during drug superfusion. ANOVA was used to determine the significance of changes in junction potential amplitudes when more than one drug was applied in sequence. When ANOVA revealed a significant difference, the significance of individual differences was determined with a post hoc Tukey-Kramer multiple comparisons test. Statistical comparisons of relative changes in responses with different stimulation regimens were made by using the Wilcoxon signed-rank test. In all statistical comparisons performed, differences were considered significant if P < 0.05. All data are expressed as means ± SE.

Drugs

The drugs used in the present study were hexamethonium, hyoscine (scopolamine), nicardipine, and pyridoxal-phosphate-6-azophenyl-2′,4′-disulfonic acid (PPADS) (all from Sigma, St. Louis, MO). Granisetron and SB-204070 were kindly supplied by SmithKlineBeecham Pharmaceuticals (Middlesex, UK). SR-142801 was kindly supplied by Dr. Emonds-Alt (Sanofi Recherche, Montpellier, France). A low-Ca2+/high-Mg2+ saline was used to block synaptic transmission composed of (in mM) 118 NaCl, 4.8 KCl, 1.0 NaH2PO4, 25 NaCO3, 12 MgSO4, 11.1 D-glucose, and 0.25 CaCl2.

RESULTS

The IJPs evoked by distension in guinea pig distal colon were similar to those recorded in guinea pig ileum (4, 22, 24). Different stimuli intensities may activate different reflex pathways, and the results here pertain to reflexes excited by maximal (or near-maximal) stimuli. They were mainly composed of an initial fast hyperpolarization followed by a second slower hyperpolarization (seen as a slowing in the return to baseline; e.g., see Fig. 2). All experiments were done with nicardipine (2.5 μM) present (see MATERIALS AND METHODS). Under these conditions, no evoked EJPs were seen in the descending pathway. The time courses of the IJPs differed...
from that reported for the rat distal colon in which IJPs were mainly monophasic (5). EJPs evoked by distension were monophasic (as has been reported, e.g., see Ref. 40), and no evoked IJPs were seen in the ascending pathway. In six animals, the average resting membrane potential of circular muscle cells was 45 mV. We observed spontaneous EJPs and IJPs occurring in both ascending and descending pathways; however, IJPs were too small to be reliably analyzed. The control frequency of spontaneous EJPs was 0.26 Hz (104 events over 10 times 40-s windows, $n = 3$) with an average amplitude of 5.0 mV. Hexamethonium (200 µM, central chamber) profoundly reduced both the frequency (0.02 ± 0.01 Hz, 9 events) and the amplitude (0.8 ± 0.3 mV) of the spontaneous EJPs. After washout, there was little recovery in frequency (0.06 ± 0.01 Hz) or in amplitude (1.8 ± 0.4 mV).

The mean latencies of IJPs and EJPs from the same preparations were compared. The average latency of EJPs evoked by distension (15 mm anal, 380 ± 20 ms) was the same as that of IJPs (15 mm oral, 380 ± 20 ms); the latency for IJPs evoked by EFS (4 mm oral) was 160 ± 10 ms ($n = 6$). In another set of preparations, the latency between IJPs evoked by far and near distension was compared. IJPs evoked by far distension (30 mm oral, 440 ± 20 ms) had a significantly longer latency than that of IJPs evoked by near distension (15 mm oral, 330 ± 10 ms; $P < 0.05; n = 6$). The apparent conduction velocity was 0.14 mm/ms.

Fig. 1. Blockade of synaptic transmission from sensory neurons in ascending and descending pathways. Synaptic transmission from sensory neurons to ascending and descending interneurons was blocked with a low-Ca$^{2+}$/high-Mg$^{2+}$ solution. A: drawing depicting a 3-chambered, divided organ bath (each chamber 15 mm wide) and neural pathways for the ascending and descending reflex pathways; oral is to the left. Pathways are presumed to reside in the myenteric plexus, but some neurons, such as the sensory neurons, may also be in the submucous plexus. The classes of neuron are shown in the inset. On the far left or far right is a recording chamber in which a microelectrode was used to make recordings from circular smooth muscle. The muscle is innervated by excitatory (left) or inhibitory (right) motoneurons (short, open neurons) that are themselves innervated by interneurons (filled neurons) and sensory neurons (multipolar, open neurons). The reflex pathways were stimulated by the inflation of a balloon (at the square pulse, 5 mm diameter) from the serosal side in the central or the far chambers. Blockade of synaptic transmission in these chambers is depicted by a large X in this and the following figures. B: pooled data ($n = 6$) showing the relative amplitudes (expressed as a percentage of the control) of excitatory junction potentials (EJPs) evoked by distension. Error bars show the SE in this and the following figures. EJPs evoked by distension from either the central or far chamber were reduced by blockade of synaptic transmission (*compared with control, $P < 0.05$; 1-way ANOVA; $n = 5–6$). C: pooled data ($n = 6$) showing the relative amplitudes of inhibitory junction potentials (IJPs) evoked by distension. IJPs evoked by distension from either chamber were reduced by blockade of synaptic transmission (*compared to control, $P < 0.05$; 1-way ANOVA; $n = 6, 6$). IJPs evoked by distension in the far chamber were significantly more depressed than IJPs evoked from the central chamber (df $P < 0.05$; Wilcoxon signed-rank test).
Effect of Synaptic Blockade in the Central Chamber on Ascending and Descending Reflexes

In the ascending pathway, EJPs evoked by distension from the central chamber were greatly reduced by blockade of synaptic transmission with a low-Ca\(^{2+}\)/high-Mg\(^{2+}\) solution in the central chamber (control, 12.9 ± 1.2 mV; high Mg\(^{2+}\), 0.9 ± 0.3; P < 0.05; one-way ANOVA; n = 5; Fig. 1B); EJPs evoked by distension in the far chamber were also greatly reduced (control, 10.3 ± 1.6 mV; high Mg\(^{2+}\), 0.7 ± 0.5; P < 0.05; one-way ANOVA; n = 6). Thus no distension-sensitive neurons or ascending interneurons project orally >15 mm (the width of the chamber). This implies that drugs applied to the central chamber will act primarily at synapses between ascending interneurons in pathways excited from the far chamber.

In contrast, in the descending pathway, IJPs evoked by distension from either chamber were only reduced by approximately half during blockade of synaptic transmission in the central chamber [central control: 17.3 ± 1.0 mV; high Mg\(^{2+}\), 10.3 ± 1.2 (59% of control); Far control: 7.1 ± 0.6 mV; high Mg\(^{2+}\), 3.0 ± 0.5 (43% of control); P < 0.05; one-way ANOVA; n = 6; Fig. 1C]. IJPs evoked by distension in the far chamber were significantly more depressed than IJPs evoked by central chamber distension (P < 0.01. Wilcoxon signed-rank test). When transmission was blocked in both the far and central chambers, IJPs evoked by distension in the far chamber were not significantly more reduced than when transmission was only blocked in the central chamber. These data suggest that some distension-sensitive neurons project from the far chamber to the recording chamber, but descending interneur-
ions do not. Distension-sensitive neurons and interneurons both project from the central to the recording chamber. Note, elements of the circuitry, which can now be excluded, have been omitted from subsequent figures.

**Transmission in the Descending Inhibitory Pathway**

**Pharmacology of transmission in the central chamber.** Transmission within the central chamber was assessed. The nicotinic receptor antagonist hexamethonium (200 μM) when added to the central chamber significantly reduced the peak amplitude of IJPs evoked by central chamber distension from 11.3 ± 0.2 to 5.3 ± 0.3 mV (47%, P < 0.01, ANOVA; n = 6). Further application of a low-Ca\(^{2+}\)/high-Mg\(^{2+}\) solution in the same chamber significantly enhanced the peak amplitude of IJPs evoked by distension from 5.3 ± 0.3 to 6.3 ± 0.3 mV (119%, P < 0.01, ANOVA; n = 6, Fig. 2A). This enhancement was similar to that seen in rat distal colon (see Discussion and Ref. 5).

We tested whether ATP also plays a role in transmission at these synapses. The nonselective P\(_2\) receptor antagonist PPADS (30 μM) in the central chamber consistently caused a small reduction in IJPs evoked by distension in this chamber [control, 13.6 ± 2.1 mV; PPADS, 12.1 ± 2.3 mV (89% of control); paired t-test, P < 0.05; n = 8, Fig. 2B].

Serotonin plays an important role in the initiation of peristalsis in the guinea pig colon, and it has been reported that 5-HT\(_3\) and 5-HT\(_1\) receptors are required (see Ref. 21). We tested a combination of the selective 5-HT\(_3\) receptor antagonist granisetron and the selective 5-HT\(_1\) receptor antagonist SB-204070. This combination, in the central chamber, significantly reduced IJPs evoked by distension in this chamber [control, 19.4 ± 2.4 mV; combination, 14.4 ± 2.8 mV (74% of control); Fig. 2C]. Similarly, when granisetron or SB-204070 were tested alone, they significantly reduced the size of the IJPs [control, 19.3 ± 1.9 mV; granisetron, 16.8 ± 1.6 mV (87% of control); control, 19.2 ± 2.7 mV; SB-204070, 16.6 ± 2.2 mV (86% of control)]. The combination of granisetron and SB-204070 was not significantly more effective than either drug alone (P > 0.05, one-way ANOVA).

When distension from the far chamber was investigated, the addition of hexamethonium (200 μM) to the central chamber significantly reduced the peak amplitude of IJPs from 8.6 ± 0.5 to 5.4 ± 0.8 mV (63% of control, P < 0.05, one-way ANOVA; n = 6). Further addition of a low-Ca\(^{2+}\)/high-Mg\(^{2+}\) solution to the same chamber did not significantly alter the peak amplitude of IJPs (from 5.4 ± 0.8 to 5.5 ± 0.7 mV, P > 0.05, one-way ANOVA; n = 6, Fig. 3A).

The nature of the residual component of this reflex was investigated by using the P\(_2\) receptor antagonist, PPADS (30 μM). PPADS, added alone to the central chamber, reduced IJPs evoked by distension in the far chamber from 10.9 ± 1.9 to 7.8 ± 1.3 mV (71% of control, paired t-test, P < 0.05; n = 7, Fig. 3B). The 5-HT\(_3\) receptor antagonist granisetron (1 μM), also added alone, had no effect (control, 5.4 ± 0.6 mV; granisetron, 5.3 ± 1.6 mV; 97% of control; paired t-test, P > 0.05; n = 4, Fig. 3C).

**Pharmacology of transmission in the recording chamber.** Hexamethonium (200 μM) in the recording chamber significantly reduced the amplitude of IJPs evoked by distension in the central chamber from 10.4 ± 0.1 to 4.8 ± 0.4 mV (46% of control; P < 0.05, n = 6) and caused a small reduction in IJPs evoked by EFS in the recording chamber from 10.5 ± 0.1 to 9.4 ± 0.4 mV (90% of control; P < 0.05, n = 6). Hexamethonium (200 μM) was significantly more effective on reflex-evoked IJPs than on electrically evoked IJPs (P < 0.05, paired Wilcoxon signed-rank test, Fig. 4A).

With a low-Ca\(^{2+}\)/high-Mg\(^{2+}\) solution in the central chamber, hexamethonium (200 μM) in the recording chamber reduced the peak amplitude of IJPs evoked by distension from 9.8 ± 0.2 to 5.4 ± 0.2 mV (55% of control, n = 6, Fig. 4B).

In a separate series of experiments, PPADS (30 μM) in the recording chamber reduced IJPs evoked by distension in the central chamber from 21.4 ± 1.3 to 18.4 ± 1.2 mV (86% of control, P < 0.05, n = 6, Fig. 4C). PPADS did not change IJPs evoked by EFS in the recording chamber (control, 17.7 ± 1.0 mV; PPADS, 18.3 ± 1.2 mV) or by distension in the far chamber (control, 12.3 ± 1.3 mV; PPADS, 12.7 ± 1.9 mV).

Granisetron (1 μM) added to the recording chamber caused a significant increase in the IJPs evoked by EFS in the recording chamber (control, 17.1 ± 1.5 mV; granisetron, 23.6 ± 1.9 mV; 138% of control; P < 0.05, Fig. 4D). Granisetron increased the amplitude of IJPs evoked by distension in the central chamber from 18.4 ± 2.0 to 20.8 ± 2.6 mV (113% of control) and caused an increase in IJPs evoked by distension in the far chamber (control, 14.0 ± 2.1 mV; granisetron, 15.5 ± 2.4 mV; 111% of control). These unexpected enhancements were not significant when assessed with a two-tailed t-test (P = 0.08, 0.37, respectively).

**Transmission in the Ascending Excitatory Pathway**

**Pharmacology of transmission in the central chamber.** Transmission within the central chamber was assessed. Hexamethonium (200 μM) in this chamber reduced EJPs evoked by distension in the central chamber from 16.2 ± 0.8 to 12.5 ± 1.1 mV (77% of control, P < 0.05; n = 6; Fig. 5A). The nonselective muscarinic receptor antagonist hyoscine (1 μM) did not reduce the EJPs nor did it potentiate the effect of hexamethonium when used in combination [control, 15.7 ± 0.9 mV; hyoscine, 14.5 ± 1.1 mV (93% of control); control, 15.7 ± 1.1 mV; hyoscine + hexamethonium, 11.8 ± 1.6 mV (75% of control), P > 0.05, one-way ANOVA; n = 6]. Similarly, the neurokinin 3 (NK\(_3\)) receptor antagonist, SR-142801 (1 μM) in combination with hexamethonium did not cause any greater change in the EJP amplitude than hexamethonium alone [control, 16.4 ± 1.4 mV; SR-142801 + hexamethonium, 13.1 ± 1.6 mV (80% of control); P > 0.05, one-way ANOVA; n = 6].

The combination of granisetron (1 μM) and SB-204070 (1 μM) in the central chamber caused a small, but significant, reduction of EJPs evoked by distension in this chamber [control, 16.9 ± 1.4 mV; combination, 14.2 ± 2.0 mV (84% of control); Fig. 5B]. In contrast, granisetron alone did not significantly reduce the EJPs [control, 17.2 ± 1.4 mV; granisetron, 15.5 ± 1.3 mV (90% of control)]. SB-204070 alone significantly reduced EJPs due to distension [control, 16.7 ± 1.5 mV; SB-204070, 14.9 ± 1.8 mV (89% of control)], and this reduction was virtually identical to that seen with granisetron and SB-204070 combined.

Transmission through the central chamber was assessed when EJPs were evoked by distension in the far chamber. These EJPs were abolished by hexamethonium (200 μM) in the central chamber (control, 12.1 ± 1.4; hexamethonium, 0 ± 0; P < 0.05; n = 5, Fig. 6A).
Pharmacology of transmission in the recording chamber. Hexamethonium (200 μM) in the recording chamber reduced EJPs evoked by distension in the central chamber from 17.2 ± 0.4 to 6.7 ± 1.0 mV (39% of control, *P < 0.05; one-way ANOVA; n = 6). When synaptic transmission was also blocked in the central chamber, the IJP amplitude did not change compared with Hex alone, though it was still different from control (*compared with control, *P < 0.05; 1-way ANOVA; n = 6). B: PPADS (30 μM) in the central chamber reduced IJPs evoked by distension in the far chamber to 71% of control (*compared with control, *P < 0.05; n = 7), whereas in a separate series of experiments (C), granisetron (1 μM) in the central chamber had no effect on IJPs evoked by distension (97% of control; *P < 0.05; n = 4).

DISCUSSION

A major finding of the present study was that distension-sensitive neurons in the guinea pig distal colon show a distinct polarity with anal projections of >30 mm in length but oral projections, if present, of 15 mm or less (for summary, see Fig. 7). In addition, nicotinic transmission played a surprisingly modest role in transmission to inhibitory or excitatory motor neurons and from sensory neurons to ascending interneurons but was the major component of transmission at the remaining synapses in the ascending and descending pathways.

Projection Lengths of Neurons Within the Reflex Pathways

Projections of distension-sensitive neurons. Synaptic blockade in the central chamber greatly reduced ascending excitatory reflexes evoked in this chamber as it does in the ileum (24), demonstrating that, if there are ascending distension-sensitive neurons in the distal colon, they project for less than the width of the chamber (15 mm). In contrast, descending...
inhibitory reflexes were only reduced by half, even when synaptic transmission was blocked in both the far and central chambers, suggesting that some distension-sensitive neurons have long axons that project anally for \( \geq 30 \) mm. Identical methods have revealed long anal projections of distension-sensitive neurons in the guinea pig ileum (4, 22, 24) and in rat colon (5), although in the ileum, these projections appeared to be substantially shorter (\( \leq 15 \) mm). In the ileum < 10% of the descending inhibition evoked by stimulation in the far chamber persists after synaptic blockade in the central chamber (e.g., 22), whereas in the colon, we have found the resistant response is approximately five times as large.

**Projections of interneurons.** The ascending interneurons were found to have an effective projection length of no greater than the width of the central chamber (i.e., 15 mm) within which synaptic transmission had been blocked. This is similar to what is seen in the ileum (8) but is surprising in the colon, because some ascending interneurons have projections that appear considerably longer in lesion studies (34).

Blockade of synaptic transmission in the central chamber also indicated that descending interneurons play as important a role in the inhibitory reflex pathway in the colon as they do in the ileum. However, the reduction in IJP amplitude was no different when synaptic transmission was blocked simultaneously in the far and central chambers. This indicates that the effective projection length of the descending interneurons is similar to that of ascending interneurons, ~15 mm or less. In contrast, tracing studies of the descending interneurons in the ileum have found the different classes of descending interneurons have projections of \( \geq 120 \) mm (8, 32).

**Nature of the distension-sensitive neurons.** There is still some discussion over the identity of the sensory elements in the intestine. The multipolar AH/Dogiel type II neurons are clearly sensitive to some stimuli (e.g., maintained stretch, certain chemicals), but this does not preclude other enteric neuronal types or extrinsic sensory nerves (e.g., vagal) from participating in reflexes evoked by these or other stimuli (e.g., for review, see Refs. 7 and 14). Indeed, in the rat colon, the
Extrinsic sensory nerves appear to be solely responsible for reflexes evoked by distension (17), whereas Spencer et al. (40, 41, 43) have recently shown that a subset of neurons with the morphology of ascending interneurons in the myenteric plexus of the guinea pig colon respond directly to maintained increases in length.

In the present study, the identity of the distension-sensitive neurons is unclear. The functional projections were asymmetrical, with long descending projections but no, or very short, ascending projections. Similar projections have been found for distension-sensitive Dogiel type II neurons (9) in the guinea pig ileum. Furthermore, the projections of extrinsic nerves would be predicted to be symmetrical, with equal lengths in the oral and anal direction, which suggests that the asymmetrical sensory pathways seen here are of intrinsic origin (i.e., an enteric neuron). That the long pathways were only descending tends to rule out a simple participation of any distension-sensitive ascending interneurons (40). In guinea pig ileum, the firing of AH/Dogiel type II neurons during maintained stretch is blocked by nicardipine (27), but firing in response to rapid mechanical distortion of their terminal is not (26). In contrast, the firing of some neurons with the morphology of ascending interneurons in the distal colon during maintained distension is not blocked by nicardipine or a 0.25 mM Ca\(^{2+}\)/12 mM Mg\(^{2+}\) solution (41). Finally, studies of the projections of AH/Dogiel type II neurons in distal colon (e.g., see Refs. 30 and 44) reported no long descending projections; however, our data indicate these projections are easy to overlook and are indeed found in the distal colon (J. C. Bornstein, unpublished observations).

Nicotinic Transmission plays a Major Role in Distension-Evoked Ascending and Descending Reflexes

Fig. 5. Blockade of neurotransmission from sensory neurons to ascending interneurons. A: Hex alone and in combination with a muscarinic and an NK\(_{3}\) receptor antagonist SR-142801 (1 \(\mu\)M) was tested in the central chamber (see inset in B). Hex (200 \(\mu\)M) in the central chamber reduced EJPs evoked by distension to 77% of control (\(*\) compared with control, \(P < 0.05\); \(n = 6\)). The nonselective muscarinic receptor antagonist hyoscine (Hyo) did not reduce the EJPs (93% of control) nor did it potentiate the effect of Hex (75% of control). Similarly, SR-142801 did not alter the reduction caused by Hex (80% of control). B: pooled data showing the relative amplitudes (expressed as a percentage of the control) of EJPs evoked by distension. EJPs were reduced by Hex alone (* compared with control, \(P < 0.05\); 1-way ANOVA; \(n = 6\)), but not by hyoscine alone (\(n = 7\)). EJPs were not significantly more depressed when hyoscine (\(n = 6\)) or SR-142801 (\(n = 6\)) were combined with Hex. Selective blockade of 5-HT\(_{3}\) receptors by granisetron (1 \(\mu\)M) and 5-HT\(_{4}\) receptors by SB-204070 (1 \(\mu\)M) were compared. EJPs were significantly reduced by SB-204070 (89% of control) but not by granisetron (90% of control). The combination of granisetron and SB-204070 caused a significant reduction of EJPs (84% of control), but this reduction was not significantly more effective than granisetron or SB-204070 alone (\(P > 0.05\); 1-way ANOVA).
ascending pathway, transmission in the stimulus chamber should be primarily from sensory neurons to ascending interneurons. When the nicotinic receptors were blocked in this chamber, the EJPs were reduced by 30%. Thus some transmission was via nicotinic receptors, but the majority was through nonnicotinic receptors. In the guinea pig ileum, non-nicotinic transmission from sensory neurons to ascending interneurons is blocked by a combination of muscarinic and NK3 receptor antagonists (22, 24, 47). However, the nonnicotinic component of transmission in the distal colon is resistant to both of these receptor antagonists. It appears that transmission from sensory neurons is mixed, with ACh and another unidentified transmitter each playing a role. Alternatively, transmission from sensory neurons may be exclusively nonnicotinic, with the apparent nicotinic component being due to ascending interneurons with synapses in the stimulus chamber. Because transmission between ascending interneurons is unambiguously nicotinic, blockade of such synapses would appear as partial blockade of the total response. In either case, it is clear that transmission from the sensory neurons in the ascending pathway is largely via nonnicotinic receptors.

Fig. 6. Blockade of neurotransmission between ascending interneurons and to excitatory motor neurons. A: distension in the far chamber. There was an average latency of 380 ± 20 ms from the onset of the inflation to the onset of the EJP (see RESULTS). Hex (200 μM) in the central chamber abolished EJPs evoked by distension in the far chamber (*compared with control, \( P < 0.05; \ n = 5 \)). B: comparison between the effect of the nicotinic receptor antagonist Hex (200 μM) and the NK3 receptor antagonist SR-142801 recording chamber on EJPs evoked by distension in the central chamber (see inset). Original voltage traces (left) and pooled data (right; \( n = 6 \)) showing the relative amplitudes (expressed as a percentage of control) of EJPs. Hex (200 μM) in the recording chamber reduced EJPs evoked by distension in the central chamber to 39% of control (*compared with control, \( P < 0.05 \); 1-way ANOVA; \( n = 6 \)). Hex combined with SR-142801 (1 μM) in the recording chamber reduced EJPs to 48% of control (*compared with control, \( P < 0.05 \); 1-way ANOVA; \( n = 6 \)), but was not any more effective than Hex alone (\( P > 0.05 \); 1-way ANOVA).

Fig. 7. Summary of the major neurotransmitter/receptor combinations in the reflex pathways. A schematic of the combined circuitry for both ascending and descending pathways excited by distension (similar to that shown in Fig. 1). The populations of neurons present and the major transmitter/receptor combinations were revealed by pharmacological analysis. Only those elements of the circuitry that were clearly defined in the present study are shown. The dotted lines from the sensory neuron in the center represent connexts that were present, but were not directly tested pharmacologically. CM, circular muscle.
In the descending inhibitory pathway, hexamethonium in the central chamber depressed reflexes evoked in this chamber to the same extent as synaptic blockade, indicating that the residual response in hexamethonium was due to anally projecting axons of distension-sensitive neurons. Thus nicotinic transmission accounts for all transmission from sensory neurons to descending interneurons in the guinea pig distal colon. A similar situation exists in the rat distal colon (5). However, only a minor role for nicotinic or muscarinic receptors has been identified in the guinea pig ileum with a large component of transmission unaccounted for (22).

Although transmission from the sensory neurons to descending interneurons appeared to be purely nicotinic, there was a small effect of blocking purinergic or serotonergic receptors. Both effects were small, which suggests these transmitters do not have an important physiological role between sensory neurons and interneurons. Serotonin may act between the enterochromaffin cells in the mucosal epithelium and the sensory nerve terminals that are nearby (3, 36, 37), as may be purines, because mucosally released ATP excites sensory nerve terminals and so, may contribute to reflexes (1, 2, 11).

Transmission between interneurons. In the ascending pathway, hexamethonium abolished transmission through the central chamber indicating that the distension-evoked reflex is exclusively carried by nicotinic transmission. In the descending inhibitory pathway, hexamethonium significantly depressed transmission through the central chamber, an effect identical to that of blocking synaptic transmission in this chamber. Thus transmission between descending interneurons may be entirely via nicotinic receptors, with the hexamethonium-resistant response being due to the long descending sensory neurons. Together, these data indicate that all transmission between the ascending and descending interneurons is via nicotinic receptors in the guinea pig distal colon. The rat colon also shows purely nicotinic transmission between descending interneurons (5). In guinea pig ileum, transmission between the ascending interneurons is purely nicotinic (24), but there is a large nonnicotinic component between descending interneurons (22–24) that may be purinergic (46). In the present study, we also found a small purinergic component to transmission between descending interneurons, but this was not additive with the nicotinic component. Thus any functional purinergic synapses may be in series with the nicotinic synapses in the descending inhibitory pathway of the colon.

Transmission to motor neurons. In the ascending pathways, the excitatory motor neurons in the recording chamber receive input only from ascending interneurons. Input was predominantly via ACh acting at nicotinic receptors, with a residual nonnicotinic component that was not due to tachykinins acting at NK3 receptors. In contrast, input to excitatory motor neurons in guinea pig ileum is partially nicotinic and partially due to tachykinins acting at NK3 receptors (22, 24).

In the descending inhibitory pathway, nicotinic transmission accounts for half of the input to inhibitory motor neurons. When synaptic transmission was blocked in the central chamber and distension was applied in this chamber so that activity entering the recording chamber was only via long descending sensory neurons, nicotinic transmission still accounted for half the input to the motor neurons. This suggests that input to inhibitory motor neurons from both long descending sensory neurons and descending interneurons is approximately half nicotinic and half via an unknown transmitter. In rat colon, input to inhibitory motor neurons from either descending pathway is purely nicotinic (5), but in guinea pig ileum, input is almost completely nonnicotinic (22), with input from descending inter-neurons being wholly purinergic and that from sensory neurons via an unidentified transmitter (4).

PPADS in the recording chamber caused a small reduction of IJP evoked by central distension but did not reduce IJP evoked by far chamber distension. Although the reduction to central distension was statistically significant, taken together, these data suggest that, if P2 receptors play a role in transmission to inhibitory motor neurons in guinea pig distal colon, it is a small one.

The nonnicotinic input to the inhibitory motor neurons was not due to 5-HT acting at 5-HT1 receptors. Blockade of 5-HT3 receptors in the recording chamber increased the amplitude of electrically evoked IJPs, and there was a trend for reflex-evoked IJPs to also be enhanced. Granisetron may block a concurrent descending excitatory reflex that interferes with the IJPs; 5-HT1 receptors are important in the descending excitatory pathway of the guinea pig ileum (35).

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