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Fast and slow synaptic potentials produced in a mammalian sympathetic ganglion by colon distension

(visceral afferent/inferior mesenteric ganglion/noncholinergic)

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ABSTRACT **Radial distension of the large intestine produced a slow depolarization in a population of neurons in the inferior mesenteric ganglion of the guinea pig. The slow potentials often occurred simultaneously with cholinergic fast potentials [(excitatory postsynaptic potentials (EPSPs)] yet persisted in the presence of nicotinic and muscarinic cholinergic antagonists when all fast EPSPs were absent. The amplitude of the distension-induced noncholinergic slow depolarization increased with increasing distension pressure. For distensions of 1-min duration at pressures of 10–20 cm of water, the mean depolarization amplitude was 3.4 mV. The slow depolarization was associated with an increase in membrane resistance, and prolonged periods of colon distension resulted in a tachyphylaxis of the depolarization. Desensitization of ganglion cells to the peptide substance P attenuated the distension-induced slow potential by an average of 49% ± 17%. Thus, two colonic mechanosensory afferent pathways converge on principal ganglion cells in the inferior mesenteric ganglion: one was previously described to be mediated by acetylcholine, and the other is described here, whose transmitter remains to be determined but which preliminary evidence suggests is mediated in part by substance P. The noncholinergic afferent pathway may enhance the intestinal inhibitory reflex mediated by cholinergic mechanosensory afferent input to the abdominal prevertebral sympathetic ganglia.**

In the prevertebral sympathetic ganglia, two general types of postsynaptic potential are recorded intracellularly. These potentials can be differentiated by their time course and the transmitters that mediate them. Fast excitatory postsynaptic potentials (EPSPs) can be evoked with single nerve shocks and are mediated by acetylcholine acting at nicotinic receptors. Slow EPSPs, elicited by repetitive nerve stimulation, are not mediated by acetylcholine (1); rather, other putative neurotransmitters have been implicated for the slow EPSP, including serotonin (2), substance P (SP) (3–5), and vasopressin (6, 7). Both fast and slow potentials can be evoked by stimulation of preganglionic or postganglionic nerves. The fibers activated by stimulation of the latter could be of two kinds: (*i*) branches of primary sensory neurons that traverse the ganglia (8, 9) and (*ii*) afferent neurons located in the gastrointestinal tract that project to the ganglia (8, 10–12). A cholinergic mechanosensory pathway to the prevertebral ganglia from the colon of the guinea pig has been described (13–17). Activation of this pathway by distension of the colon results in an increase in the frequency and amplitude of cholinergic EPSPs in ganglionic neurons. This pathway has been shown to mediate the afferent limb of a mechanosensitive intestinal reflex (16), in which distension of a segment of colon inhibits motility in an adjacent segment (10). It is not known whether the gastrointestinal mechanosensory pathway also comprises noncholinergic fibers; indeed, it remains to be determined whether noncholinergic slow EPSPs even occur physiologically.

We report here the discovery of a noncholinergic sensory pathway that projects from the distal colon to the inferior mesenteric ganglion (IMG) of the guinea pig. This pathway, activated by colon distension, produces noncholinergic slow depolarizations resembling nerve-evoked slow EPSPs in sympathetic ganglion cells. Often, distension of the colon produced both an increase in cholinergic EPSPs and a slow depolarization, suggesting a simultaneous action of two different neurotransmitters on a single ganglion cell. The noncholinergic mechanosensory input to the IMG may play a significant role in mediating the intestinal inhibitory reflex induced by distension.

MATERIALS AND METHODS

IMG–Colon Preparation. The IMG and an attached segment (3.5–4.0 cm) of distal colon were excised from male guinea pigs (150–400 g) and mounted in separate compartments of a two-compartment recording bath. The intermediate mesentery containing the lumbar colonic nerves, which communicate between the colon and the IMG, was draped over the barrier separating the two compartments and covered with moist gauze to prevent drying by exposure to air. Both compartments were perfused with oxygenated Krebs solution (35–37°C). In some experiments, both nicotinic and muscarinic cholinergic synaptic transmission was blocked in the IMG by superfusion through the ganglion compartment of hexamethonium bromide (100 μM) and atropine (2 μM). The aboral end of the colon segment was ligated, and the oral end was fastened to a fluid injector/pressure transducer assembly used to distend the colon. The colonic intraluminal pressure was quantitated in centimeters of water ascending in an open vertical manometer placed in parallel with the fluid injector.

Intracellular Recording. Membrane potential of IMG neurons was monitored intracellularly with glass microelectrodes filled with 3 M KCl and having tip resistances of 30–80 MΩ. To measure membrane resistance, constant anodal current pulses were injected into the cell through the recording microelectrode. The current pulses gave rise to hyperpolarizing potential deflections, changes in the amplitude of which served as a measure of changes in membrane resistance. Nerve trunks were stimulated with bipolar platinum electrodes. Electrically evoked slow EPSPs were evoked with hypogastric nerve stimulation and not lumbar colonic nerve stimulation so as not to alter colonic contractility. However, in separate experiments stimulation of either nerve gave qualitatively similar slow depolarizations.

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Abbreviations: EPSP, excitatory postsynaptic potential; IMG, inferior mesenteric ganglion; SP, substance P.

RESULTS

Simultaneous Fast and Slow Potentials. In the absence of cholinergic antagonists, 78% (18 of 23) of the ganglion cells impaled exhibited asynchronous cholinergic fast EPSPs that arose continuously from spontaneous contractions of the colon segment in the nondistended state. When the colon was distended (5–20 cm of H_2O), the frequency and amplitude of fast EPSPs increased in proportion to the intraluminal pressure (Fig. 1*A*). Fig. 1*A* also shows the depolarization from resting membrane potential that often accompanied the increased cholinergic activity. Then cholinergic synaptic transmission was blocked by superfusing hexamethonium bromide (100 μM) over the ganglion; after approximately 2 min of superfusion, all fast EPSPs were abolished. Atropine (2 μM) was also administered to prevent the possibility of a muscarinic action of acetylcholine, although no muscarinic synaptic potentials have been described in this ganglion. Subsequent distension of the colon segment generated a slow depolarization, now in the absence of fast EPSPs (Fig. 1*B*). Thus, the fast and slow potentials are apparently differentially mediated. In 28% (5 of 18) of IMG neurons that were studied in the absence of cholinergic antagonists and that had asynchronous fast EPSPs, colon distension enhanced cholinergic activity without producing any membrane depolarization. Conversely, in only 1 of 5 cells did colon distension produce a slow depolarization in the absence of any recorded cholinergic EPSPs.

Characteristics of the Distension-Induced Slow Potential. Experiments to characterize the noncholinergic slow potential evoked by colon distension were performed in the absence of cholinergic synaptic activity, which was blocked by cholinergic antagonists (described above). In these experiments, distension of the colon segment produced slow depolarizations in 43 of 120 cells (36%) tested in 27 ganglia. The amplitude of depolarization increased with the distension pressure until a pressure was reached at which no further depolarization occurred; this maximum-response pressure varied among cells, ranging from 15–20 cm of H_2O. The depolarization usually began within seconds after imposing the distension stimulus. For distensions of 1 min duration at 10–20 cm of H_2O, slow depolarizations reached a peak amplitude (mean ± SEM) of 3.4 ± 0.3 mV (1.0–7.2 mV) at approximately 1 min, while total duration of the depolarization was 108 ± 7 s (N = 21 cells). Occasionally, the depolarization was sufficient to initiate firing of action potentials. Distending the segment for longer periods of time (2–10 min) increased the duration of the depolarization but not the mean amplitude (N = 9). In eight of nine cells, prolonged periods of colon distension resulted in a depolarization followed by a partial or full repolarization (58–100%) to the initial membrane potential within 5 min.

In seven of nine cells, the slow depolarization was associated with an increase in membrane resistance, whereas in two cells resistance remained unchanged. The mean increase in membrane resistance was 24 ± 5% compared to predistension values. An example of the distension-induced increase in resistance is shown in Fig. 2. In 2*A*, the voltage (bottom trace) clearly shows a depolarization in response to colon distension (top trace). At the peak of the depolarization, the potential

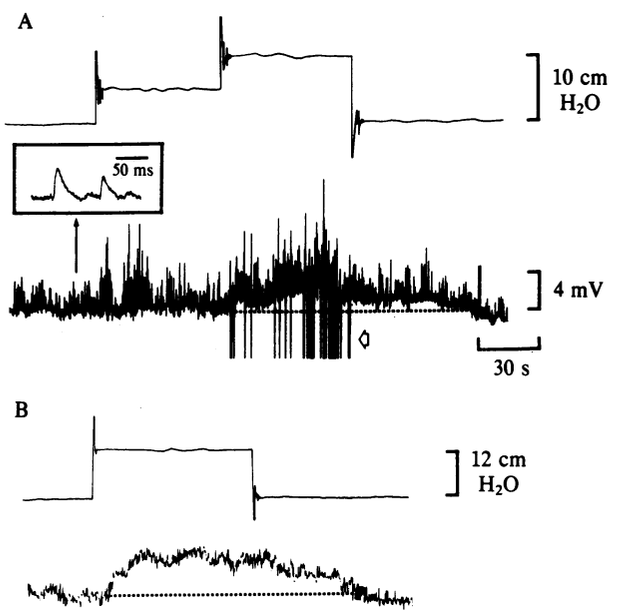

FIG. 1. Fast and slow potentials in the guinea pig IMG produced by distension of the distal colon. In *A* and *B*, upper traces are intraluminal pressure in cm of H_2O displaced in an open vertical manometer; upward deflections indicate colonic distension. Lower traces are intracellular recordings in an IMG cell. (*A*) Distension of the distal colon produced a pressure-dependent increase in frequency and amplitude of fast cholinergic EPSPs. Fast EPSPs in *A* appear as upward deflections at this slow sweep speed but are shown expanded in *Inset*. At the higher distension pressure, some EPSPs summated to action potentials. The upstrokes of the action potentials were largely cut off at the slow sweep speed, but their afterhyperpolarizations (downward deflections) are evident (open arrow). Note that at a pressure of 10 cm of H_2O, the membrane depolarized 4 mV. (*B*) With cholinergic fast EPSPs blocked by hexamethonium (100 μM), colon distension elicited a slow depolarization of several mV. Traces in *A* and *B* were recorded from the same cell.

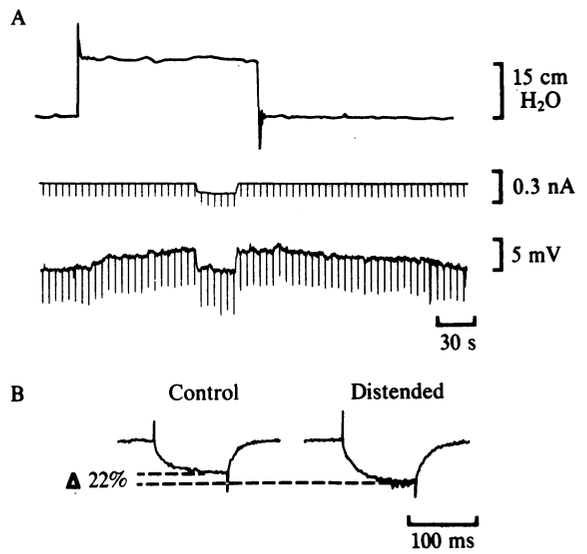

FIG. 2. Increase in membrane resistance during the depolarization produced by colon distension. The Krebs solution superfusing the ganglion contained hexamethonium (100 μM) and atropine (2 μM). (*A*) The top trace is intraluminal pressure, the middle trace is current, and the bottom trace is the voltage, recorded intracellularly in an IMG neuron. Constant anodal current pulses (0.12 nA, 100 ms) were injected into the cell through the recording electrode, producing corresponding hyperpolarizing potential deflections in the voltage trace. As the membrane depolarized during colon distension, the potential was manually clamped briefly to its initial level by injection of dc hyperpolarizing current into the cell. The hyperpolarizing deflections during the period of clamp have larger amplitudes than those prior to distension, indicating that an increase in membrane resistance occurred during distension. (*B*) Two hyperpolarizing potential deflections from *A* are shown expanded at a faster sweep speed. The "control" deflection was one recorded prior to colon distension, and the one labeled "distended" was recorded during the period of distension when the membrane potential was clamped to its initial level. Comparison of their amplitudes reveals a 22% increase in resistance caused by colon distension.

was manually clamped to the initial level to annul any possible effect of membrane rectification. The resistance, as measured by the amplitude of the hyperpolarizing potentials in the voltage trace, was increased during the period of distension compared to the period prior to distension. This is better seen in Fig. 2*B*, where hyperpolarizing potentials before (control) and during colon distension (under current clamp) are compared at a faster sweep speed. As shown, the increase in resistance for this cell was 22%. In nine cells (nine preparations), repeated distensions (up to six) produced correspondingly slow depolarizations, demonstrating that the distension necessary to produce the responses did not damage the colon or the sensory nerves. Tetrodotoxin (0.3 μM) superfused over the ganglion prior to distension reversibly blocked the slow depolarization (N = 4).

SP Desensitization. Several of the peptides that are localized within nerve fibers in prevertebral ganglia depolarize ganglion cells, including SP (3–5), vasoactive intestinal polypeptide (18), vasopressin (6), and cholecystokinin (M. A. Schumann and D.L.K., unpublished data). The membrane effects associated with the distension-induced slow potential are identical to those produced by these peptides and by repetitive nerve stimulation. Fig. 3 illustrates this point for SP, showing a distension-induced slow potential, a slow EPSP, and a depolarization produced by SP. Because the distension-induced depolarizations are transmitted to the IMG via the lumbar colonic nerves, the hypogastric nerves were stimulated in these preparations to preserve the integrity of the IMG with the colon and to avoid altering colonic contractility. Previous studies have shown that both the lumbar colonic nerves and the hypogastric nerves contain SP of central origin (19, 20). Each of these depolarizations is also associated with an increase in membrane resistance (not shown). Because the depolarizing effects of SP are the most fully characterized, we performed distensions before and after desensitization to SP as a preliminary determination of whether SP is involved in mediating the distension-induced slow potentials. Desensitization of membranes to a particular neurotransmitter attenuates any further effect of that neurotransmitter (21). In each of three cells in which a slow depolarization was elicited by distension, SP (1 μM) was then superfused over the IMG, resulting in a depolarization of 4–10 mV and subsequent return to near the original membrane potential after 6–9 min of superfusion. At this time the cell was considered to be desensitized to SP. In two of the three cells, SP desensitization completely abolished the slow depolarization (Fig. 4), while in the other cell the amplitude was reversibly attenuated by 38% from control. In a previous study (22), we determined that SP desensitization does not alter the amplitude of cholinergic fast EPSPs evoked by presynaptic nerve stimulation. Thus, desensitization specifically attenuates the noncholinergic depolarization without affecting cholinergic transmission.

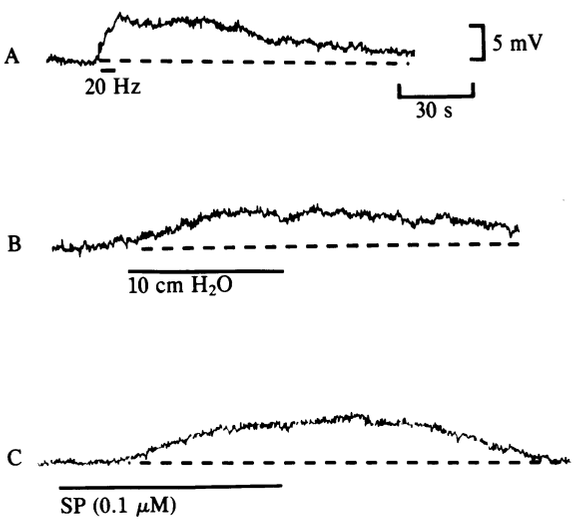

FIG. 3. Slow excitatory responses recorded from neurons of the guinea pig IMG. All recordings were made with hexamethonium (100 μM) and atropine (2 μM) in the superfusing Krebs solution. (*A*) A slow EPSP elicited by repetitive stimulation of the hypogastric nerve at 20 Hz for 5 s. (*B*) A slow excitatory potential resulting from distension (10 cm of H_2O) of the distal colon for a period of 1 min. (*C*) A slow depolarization produced by superfusion of the ganglion with SP (0.1 μM) for 90 s. In each trace the solid horizontal line indicates the duration of the applied stimulus or superfusion, and the dashed line indicates the apparent resting membrane potential. Traces in *A*, *B*, and *C* were recorded in different cells.

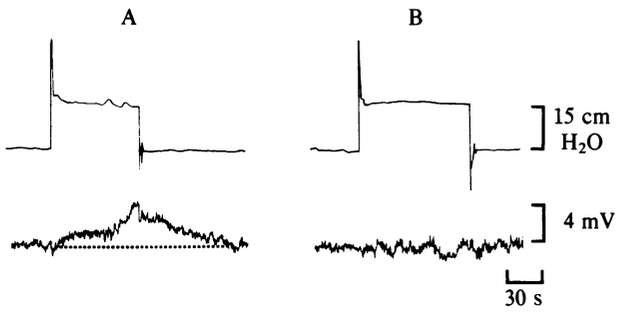

FIG. 4. The effect of SP desensitization on the noncholinergic slow potential produced by colon distension. This experiment was carried out with hexamethonium (100 μM) and atropine (2 μM) in the perfusing Krebs solution. Desensitization to SP occurred after prolonged exposure of the IMG to SP (1 μM) (see text). Upper traces indicate colonic intraluminal pressure; lower traces are intracellular recordings from the same IMG neuron. (*A*) Control distension, which resulted in a slow depolarization of 4 mV. (*B*) Distension after SP desensitization failed to produce a slow depolarization. Data from this and other cells suggest a possible role for SP in the transmission of the distension-induced slow depolarization.

DISCUSSION

We have demonstrated the existence of a noncholinergic mechanosensory pathway from the distal colon to the IMG in guinea pigs. This afferent pathway, which travels along the lumbar colonic nerve, is responsive to changes in colonic intraluminal pressure. Activation of these afferents by distension of the colon produces in a population of IMG neurons slow depolarizations that are resistant to cholinergic blockers. The depolarizations were indeed synaptic responses and not a mechanical artifact of distension because they were abolished in the presence of tetrodotoxin. Slow depolarizations produced by colon distension were observed previously (23), accompanied by asynchronous fast EPSPs. However, the authors did not determine the noncholinergic nature of the slow depolarizations but rather suggested that they arose due to summation of fast EPSPs.

The cholinergic mechanosensory pathway from the distal colon was previously deduced to arise from mechanoreceptor neurons originating in the wall of the colon (13–15). The processes of these afferents terminate on a population of principal sympathetic neurons in the IMG (24). Our experiments indicate that many IMG cells are innervated by both noncholinergic and cholinergic mechanosensory pathways. In some cells, however, only the noncholinergic depolarization could be elicited by distension, while in others only cholinergic EPSPs occurred. Thus, the two mechanosensory pathways have innervation patterns that overlap somewhat in the ganglion but are not identical. Previous estimates of the percentage of IMG neurons receiving spontaneous cholinergic afferent input from the large intestine ranged from 79%

(14) to 100% (13), whereas in the present study 36% of neurons tested received noncholinergic afferent input. These data suggest that a greater proportion of IMG cells are innervated by cholinergic rather than by noncholinergic colonic afferent fibers.

Histological (9, 11) and electrophysiological (25, 26) studies suggest that prevertebral ganglia receive afferent innervation from viscera other than the colon through other nerve tracts. This sensory information is integrated with preganglionic efferent input in the regulation of visceral organ function (15, 25–28). The results of this study indicate that the IMG integrates not just cholinergic information but also signals conveyed by another neurotransmitter. Although evidence for this comes only from our studies of the colon, the possibility exists that noncholinergic inputs arrive at the IMG from other abdominal viscera as well.

The transmitter of the distension-induced noncholinergic potentials remains to be determined; however, our experiments with SP desensitization suggest that this peptide may be involved. Desensitization selectively attenuates the noncholinergic potentials without reducing cholinergic fast EPSPs (22). Furthermore, there is no cross-desensitization between SP and vasopressin (6), another putative peptide neurotransmitter in sympathetic ganglia, and not all slow EPSPs are attenuated by substance P desensitization. SP in the IMG is localized in collateral terminals of primary sensory neurons of the C-fiber type, which originate in the dorsal root ganglia (19, 20, 29). Many of their peripheral axons course through the IMG and on to the distal colon (8, 9). Perhaps the noncholinergic afferent pathway is activated by intramural mechanoreceptors of primary sensory neurons. Because desensitization to SP did not completely abolish all of the distension-induced noncholinergic potentials, we cannot exclude the participation of the other neuropeptides in mediation of the slow synaptic potentials. Indeed, in previous experiments (22) the persistence of slow EPSPs after treatment with capsaicin suggested that transmitters other than SP may be involved in their mediation or in the mediation of some of them.

It should be mentioned that we used desensitization to SP in our experiments rather than SP antagonists because of the poor efficacy of the antagonists in blocking the effects of SP in autonomic ganglia. Published reports (30, 31) cite only a modest blocking effect at high concentrations (1–50 μM); however, we have been unable to detect any consistent inhibition of SP responses in the IMG by the two most potent antagonists (32), [D-Arg^1, D-Pro^2, D-Trp^{7,9}, Leu^{11}]SP$_{1-11}$-NH$_2$ and [D-Arg^1, D-Trp^{7,9}, Leu^{11}]SP$_{1-11}$-NH$_2$ (unpublished observations).

In addition to those recorded in guinea pig prevertebral ganglia, fast and slow synaptic potentials have been recorded in other ganglia by using nerve-stimulation techniques. In both the rabbit superior cervical ganglion (33) and amphibian paravertebral sympathetic ganglion (34, 35), preganglionic nerve stimulation elicits a fast EPSP, followed by a slow inhibitory potential (IPSP), a slow EPSP, and, finally, a late slow EPSP. In the present study, however, fast and slow potentials were produced by a *physiologic* stimulus rather than by electrical stimulation of an isolated nerve.

The simultaneous action of acetylcholine and a noncholinergic transmitter in the IMG is significant in terms of broadening our classical concept of transmitter action. Indeed, this may be a physiologic demonstration of how one of a pair of transmitters "enables" or amplifies the effectiveness of the other, as proposed by Bloom (36). In the IMG, the transmitter of the noncholinergic afferent pathway amplifies the cholinergic excitation of the postsynaptic membrane. That is, the noncholinergic slow depolarization increases the likelihood that cholinergic fast EPSPs will summate to action potentials. This occurs because (*i*) the slow depolarization raises the membrane potential closer to firing threshold and (*ii*) the increased membrane resistance results in a greater voltage deflection for a given excitatory postsynaptic current. The result is an increased probability that a receiving postganglionic sympathetic neuron will conduct action potentials.

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