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An Enteric Occult Reflex Underlies Accommodation and Slow Transit in the Distal Large Bowel

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Background & Aims: **Transit of fecal material through the human colon takes ≥30 hours, whereas transit through the small intestine takes 24 hours. The mechanisms underlying colonic storage and slow transit have yet to be elucidated. Our aim was to determine whether an intrinsic neural mechanism underlies these phenomena.** ***Methods:*** **Recordings were made from circular muscle (CM) cells and myenteric neurons in the isolated guinea pig distal colon using intracellular recordings and Ca^{2+} imaging techniques. Video imaging was used to determine the effects of colonic filling and pellet transit.** ***Results:*** **Circumferential stretch generated ongoing oral excitatory and anal inhibitory junction potentials in the CM. The application of longitudinal stretch inhibited all junction potentials. N-ω-nitro-L-arginine (100 μmol/L) completely reversed the inhibitory effects of longitudinal stretch suggesting that nitric oxide (NO) inhibited interneurons controlling peristaltic circuits. Ca^{2+} imaging in preparations that were stretched in both axes revealed ongoing firing in nNOS +ve descending neurons, even when synaptic transmission was blocked. Inhibitory postsynaptic potentials were evoked in mechanosensitive interneurons that were blocked by N-ω-nitro-L-arginine (100 μmol/L). Pellet transit was inhibited by longitudinal stretch. Filling the colon with fluid led to colonic elongation and an inhibition of motility.** ***Conclusions:*** **Our data support the novel hypothesis that slow transit and accommodation are generated by release of NO from descending (nNOS +ve) interneurons triggered by colonic elongation. We refer to this powerful inhibitory reflex as the intrinsic occult reflex (hidden from observation) because it withdraws motor activity from the muscle.**

The large bowel has evolved to serve several major functions: to recover water and electrolytes from the contents of the intestinal lumen, to use bacteria to digest nutrients (in instances in which appropriate enzymes are lacking), and to prevent being tracked by scent-orientated predators and mark territories.[1] To perform these functions, transit of intraluminal contents through the human colon is slow, taking ≥30 hours, whereas transit through the small intestine takes 2–4 hours.[1–4] As intraluminal contents move down the large bowel, water and electrolytes are absorbed, causing the contents to become more viscous leading to stool formation. However, the mechanism underlying colonic accommodation and slow transit to promote absorption is unknown,[1–5] although increased segmental contractions and extrinsic sympathetic reflexes have been proposed.[1–4] Recently, an intrinsic neural mechanism involving an excess production of nitric oxide (NO) within the myenteric plexus, the ganglionated network that controls motility,[5,6] has been proposed from immunohistochemical studies to have a role in the pathology underlying slow transit constipation in the human large bowel.[7,8] However, how an excess of NO might alter transit is unknown.

We have recently proposed that there are multiple sensory neurons in the myenteric plexus of the guinea pig large intestine.[9] There appears to be 2 intrinsic sensory systems underlying peristaltic type motor patterns that are analogous to tension-sensitive Golgi tendon organs and length-sensitive muscle spindles in skeletal muscle. Activation of these systems by circumferential stretch applied to the colon generates neurally mediated peristaltic waves that are dependent on muscle tone (duration ~40–60 seconds; frequency, ~0.3/min)[10] and ongoing peristaltic reflex activity (duration, ~3 seconds; frequency, ~11/min), which is independent of muscle tone but generated by increases in the diameter of the bowel.[11–13] These 2 motor patterns appear to be generated by myenteric after-hyperpolarization (AH)-type sensory neurons, which sense muscle tension,[14] and mechanosensory S-type ascending and descending interneurons that detect changes in muscle length around the circumference of the bowel.[11,13,15] Multipolar AH neurons, which were until recently thought to be the only sensory neuron in the gut, are defined for their long-lasting AH following an action potential. Unipolar synaptic (S) neu-

Abbreviations used in this paper: CM, circular muscle; CMMP, circular muscle myenteric plexus; EJP, excitatory junction potential; FEPSP, excitatory postsynaptic potential; IJP, inhibitory junction potential; IPSP, inhibitory postsynaptic potential; LM, longitudinal muscle; L-NA, N-ω-nitro-L-arginine; PPADS, pyridoxal phosphate-6-azophenyl-2,′4′-disulfonic acid; TS, transmural stimulation.

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rons, on the other hand, receive fast excitatory synaptic input and comprise the mechanosensory interneurons and excitatory and inhibitory motor neurons.[11,15]

Recently, low-threshold extrinsic afferent mechanoreceptors in the rectum have been shown not to exhibit directional sensitivity because they respond equally to both circular and longitudinal stretch.[16] Given this finding, we wanted to determine whether peristaltic activity in the colon is affected not only by circumferential stretch but also by longitudinal stretch. We also investigated how intrinsic release of NO might affect these reflexes. We demonstrate that a third myenteric sensory neural system, which is inhibitory, is activated by colonic elongation. These mechanosensory neurons release NO within the myenteric plexus resulting in a depression of activity of mechanosensitive interneurons driving peristaltic nerve pathways activated by circumferential stretch.

Materials and Methods

Intracellular Microelectrode Recordings From Smooth Muscle

Male and female guinea pigs (200–350 g) were killed in accordance with the Animal Ethics Committee at the University of Nevada-Reno, and a segment of distal colon (15–20 mm long) was removed. The mucosa and submucosa were removed to reveal the circular muscle (CM), and the segment was threaded through greased (High Vacuum Grease; Dow Corning, Midland, MI) rubber diaphragms in 2 partitions (7 mm apart; see Figure 1*A*). Each chamber was separately perfused with warmed oxygenated Krebs' solution (36.0°C ± 0.5°C) containing nifedipine (1–2 μmol/L) to paralyze the smooth muscle.[9–12] Simultaneous microelectrode recordings were made from CM cells at both the oral and anal ends of the preparation. Preparations were stretched and pinned either in the circumferential (12 mm, twice their resting diameter)[11–13] or longitudinal directions or in both directions simultaneously. Once control activity was recorded, the preparation was then stretched and repinned in both axes, which took approximately 2–3 minutes. After a 10-minute equilibration period, microelectrodes were reinserted into CM cells at either end of the tissue. Longitudinal stretch was normalized to the slack segment length. Simultaneous intracellular recordings were made from CM cells using 2 independently mounted micromanipulators (WPI; World Precision Instruments, Inc. Sarasota, FL; model M3301R). Microelectrodes (ID, 0.5 mm) were filled with 1.5 mol/L KCl solution and had tip resistances of approximately 100–150 MΩ. Electrical signals were amplified using a dual input Axoprobe 1A amplifier (Axon Instruments, Foster City, CA) and digitized at 2–5 kHz for neuronal recordings (see below) and 500 Hz to 1 kHz for smooth muscle recordings on a PC running Axoscope software (version 8.0; Axon Instruments).

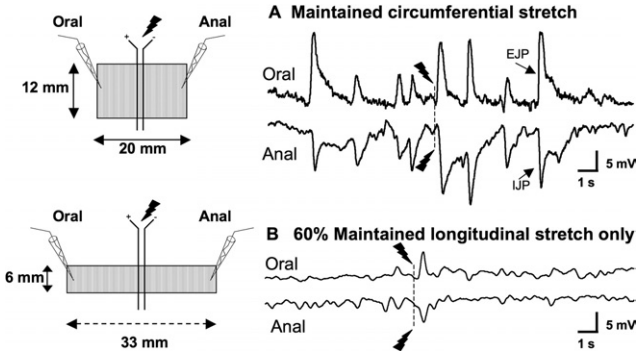

Figure 1. Effects of circumferential and longitudinal stretch. (*A*) Circumferential stretch applied to the distal colon activated ongoing peristaltic reflex activity consisting of oral excitatory junction potentials (EJPs) that were coordinated with anal inhibitory junction potentials (IJPs) in the circular muscle. Transmural nerve stimulation (transmural stimulating single pulse 0.5 ms, 30 V) applied to the middle of the preparation evoked a robust oral EJP and anal IJP. (*B*) When the same preparation was stretched only in the longitudinal axis, only low-amplitude neural activity was observed and gave only a small response to TS.

CM Myenteric Plexus Preparations

CM myenteric plexus (CMMP) preparations were prepared by removing the longitudinal muscle (LM). The LM was removed by teasing up muscle fibers at the oral end, pinching these fibers with a pair of curved forceps and peeling the LM off the preparation, in an oral to anal direction. This easily removed the LM, which often came off as wide strips of muscle, without damaging the myenteric plexus that remained intact upon the CM. The integrity of the enteric circuitry was assessed by the fact that the CM generated spontaneous coordinated oral excitatory junction potentials (EJP) and anal inhibitory junction potentials (IJP). This would not happen if there was any damage to the myenteric plexus from removing the LM.

Intracellular Microelectrode Recordings From Myenteric Neurons

The mucosa and submucosa and the LM were removed from a segment of distal colon 20 mm in length to create a CMMP preparation. The segment, which was stretched circumferentially to twice its slack width, was pinned in an organ bath with the myenteric plexus uppermost. Retaining the CM ensures that neurons in peristaltic nerve pathways are spontaneously active.[13,15] Transmural stimulating wires were placed across the oral end of the preparation approximately 10 mm from the recording site. Once a neuron was impaled, transmural stimuli (TS) were applied (0.5-ms duration, 10 Hz for 5 to 10 pulses). Standard intracellular recording techniques were used to record from myenteric neurons at the anal end of the segment.[13,15] The organ bath was separately perfused with warmed oxygenated Krebs' solution (36.0°C ± 0.5°C) containing nifedipine (1–2 μmol/L) to