Enteric Nervous System: Neuropathic Gastrointestinal Motility

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The enteric nervous system (ENS) is universally acknowledged to be an independent *brain-in-the-gut* essential for normal motility of the esophagus, stomach, small intestine and colon, as well as for smooth muscle sphincters [1–3]. As such, the enteric “mini-brain” organizes differing kinds of specialized motility in each of the functionally differentiated compartments along the digestive tract, starting in the esophagus and ending in the recto-sigmoid in humans. My perspective illustrates some of the ways in which neuropathy in the ENS accounts for various forms of disordered motility and their associated patho-physiologic symptoms.

Neural networks in the ENS are “wired” for control of propulsive motility without dependence on input–output connections with the brain or spinal cord. Independent status of the ENS is underscored by the functionally organized propulsion that can be demonstrated in the large intestine, isolated in vitro apart from any central nervous connection. Propulsive motility in colonic preparations in vitro mimics defecation and verifies the independent integrative nature of the ENS [4, 5].

Perception of the ENS as a brain-like nervous system implies that, like central nervous systems throughout the animal kingdom, the ENS is assembled in a hierarchical organization of neural mechanisms. Output from the multiple tiered hierarchy is responsible for moment-to-

moment behavior of the gastrointestinal musculature, secretory glands and blood vasculature in the operational gut. Integrated output to the musculature is observed experimentally as coordinated patterns of motility that are readily recognizable during specific digestive states (see later).

Neurons and synaptic transfer of chemical information from neuron to neuron are at the base of the hierarchically stacked tiers of ENS organization (Fig. 1). Higher up the hierarchy, synaptic “wiring” of interneurons and motor neurons in a single polysynaptic reflex circuit underlies all patterns of propulsive motility in the tubular compartments of the esophagus and small and large intestines (Fig. 2). The circuit fits the definition of a reflex in the sense that each time it is activated, the pattern of output behavior consistently occurs in the same way. In the tubular compartments of the digestive tract, stereotypic output is shortening of the longitudinal muscle coat and inhibition of the circular muscle coat in the downstream direction of propulsion and contraction of the circular muscle coat and relaxation of the longitudinal muscle coat upstream from the direction of propulsion.

Higher up in the ENS hierarchy is an intermediate organizational level consisting of neuronal pattern generators that drive the timing of repetitive activity of the propulsive reflex circuit [6]. Situated at the top of the organizational hierarchy is a library of programs for motor behaviors that emerge from the organization of the different kinds of neurons, their synaptic connections and their connectivity into microcircuits at lower levels (Fig. 3). A library, which stores neural programs for each of the motility patterns associated with different states of digestion, emerges at the top level of the ENS hierarchical order. Prof. C. Ladd Prosser, at the University of Illinois Champaign-Urbana, taught his students, including me, that

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Fig. 1 Depiction of the brain-in-the-gut as organized in a systematic hierarchy. Neuronal subcellular physiology and synaptic transfer of information from neuron to neuron are at the lowest tiers of hierarchical organization. Synaptic connectivity of neurons into a single “hardwired” polysynaptic reflex circuit, which becomes the basis for all patterns of propulsive motility and any linked secretory behavior, is an intermediate tier. Neuronal pattern generators that drive the timing of repetitive activity of the reflex circuit space are higher up the hierarchy. Near the *top tier* of neural organization are programs for behaviors that emerge from the organization of the different kinds of neurons, their synaptic connections, and their connectivity into microcircuits in the *lower tiers*. A library that stores a neural program for each of several digestive states such as postprandial mixing motility and emesis fills-out the *top tier*. Plasticity/adaptability-programmed behavior inside the specialized gut compartments is a distinguishing property of the *top tier*. Functioning of each program in the library of behaviors is reminiscent of mobile “apps” (software applications) that run on modern smart phones, tablet computers, and comparable mobile devices

acquisition of a total understanding of mechanisms at lower tiers of a central neural hierarchy does not enable prediction of the kinds of behavior that will emerge at the next higher tier or for that matter at levels of whole animal behavior. This implies that the specialized patterns of motility are not predicted by results of basic work addressing lower levels of the ENS hierarchy. On the other hand, the physiology underlying behavior seen at the whole organ level cannot be understood without basic mechanistic knowledge at the lower levels of organization.

The ENS library, in mammalian small intestine, contains programs known for at least five different patterns of motility: (1) *interdigestive state*; (2) *postprandial state*; (3) *aboral power propulsion*; (4) *emetic oral power propulsion*; and (5) *physiologic ileus*. Aside from the small bowel, formation and dynamic behavior of haustral pouches are reflections of operation of one of the motility programs in the ENS library of the colon.

Interdigestive Motor Program

The migrating motor complex (MMC) identifies the ENS-programmed small intestinal motility pattern of the interdigestive state. It is “called-up” from the program library when digestion and absorption of nutrients is complete 2–3 h after a meal. MMC behavior was first discovered by recording electrical activity with electrodes attached to the serosal surface of the intestine in conscious dogs [7, 8]. Sensors, attached to the stomach, show the MMC starting as large amplitude circumferential contractions at a rate of 3/min in the human mid-stomach. Activity starts in mid-stomach and migrates in the aboral direction into the duodenum and on through the small intestine to the ileum.

When the program is “running” in the small intestine, the MMC occupies a limited length of intestine called the activity front, which is seen to have an upper and lower boundary with absence of contractile activity on either side (physiologic ileus, see later). The activity front slowly advances; it “migrates” down the intestine at a rate that progressively slows as it approaches the ileum. Propulsion of luminal contents in the aboral direction occurs between the oral and aboral boundaries of the activity front. The circular muscle contractions inside the activity front are a reflection of the formation of the propulsive component of the “hard-wired” propulsive reflex circuit (see Fig. 2). Each propulsive wave, traveling downward in the activity front, has a propulsive and receiving segment (see Fig. 3c). Successive propulsive waves start at the oral boundary and propagate to the aboral boundary of the activity front, where they stop. Successive propulsive complexes start, on average, a short distance further in the aboral direction and propagate, on average, beyond the boundary where the previous one stopped. Thus, the entire activity front appears to migrate down the intestine, “sweeping” the lumen clean as it propagates. This function inspired Charles F. Code and Joseph H. Szurszewski of the Mayo Clinic, to name the MMC “the bowel’s housekeeper” [9, 10]. A program, termed *physiologic ileus* (see later), is in effect along the bowel oral and aboral to the upper and lower boundaries of the migrating activity front. Neither MMCs nor physiologic ileus can be found in the small intestine when the ENS is absent or non-functional.

Postprandial Motor Program

Feeding immediately terminates “running” of the MMC and starts the postprandial motor program, which is responsible for the mixing pattern of motor behavior typically seen on fluoroscopic images and magnetic resonance imaging. The mixing movements, sometimes

Fig. 2 Enteric nervous control of propulsive motility. **a** A “hard-wired” polysynaptic peristaltic reflex circuit in the enteric nervous system organizes propulsive and receiving segments that underlie all forms of esophageal and intestinal propulsive motility. When the reflex circuit is activated, excitatory musculomotor neurons to the longitudinal muscle coat and inhibitory musculomotor neurons to the circular muscle coat fire and form the receiving segment. Firing of excitatory musculomotor neurons to the circular muscle coat and suppression of firing in inhibitory musculomotor neurons to the circular muscle coat occur in the propulsive segment. **b** Reflex behavior of the longitudinal and circular muscle coats forms propulsive and receiving segments as the basis of propulsive motility. The longitudinal muscle coat in the segment ahead of the advancing intraluminal contents contracts, while the circumferential muscle layer relaxes at the same time. Simultaneous shortening of the longitudinal intestinal axis and relaxation of the circumferential muscle in the same segment results in expansion of the lumen, which

becomes a receiving segment for the forwardly moving contents. A related component of the reflex is contraction of the circular muscle in the segment behind the advancing intraluminal contents. The longitudinal muscle coat in this segment relaxes, while the circular muscle contracts, resulting in conversion to a propulsive segment that propels the luminal contents ahead into the receiving segment. **c** Propulsive behavior of the intestinal wall during activation of the “hard-wired” polysynaptic propulsive reflex in guinea pig ileum in response to distension by infusion of saline at time = xs. Shortening of the longitudinal axis and expansion of the lumen is seen in the receiving segment and contraction of the circular muscle and reduction in the diameter of the receiving segment is apparent at time = 3xs as propulsion continues and empties the lumen through the outlet tube. Wall behavior was redrawn from photographic images provided, as a courtesy, by Prof. Miako Takaki, Department of Physiology, Nara Medical University, Nara University, Japan

called *rhythmic segmentation*, were first studied by Walter B. Cannon, who invented the term based on appearance of the motility pattern on radiographic films of the small intestine after a meal [11]. Propulsive contractions of the circular muscle, which propagate only short distances, account for the segmentation appearance (see Fig. 3a). Circular muscle contractions in short propulsive segments are separated on either side by receiving segments with relaxed circular muscle and contracting longitudinal muscle, each of which reflects activation of a short “block” of the ENS polysynaptic peristaltic reflex circuit (see Fig. 2). Each propulsive segment jets the chyme in oral and anal directions into relaxed receiving segments where stirring and mixing occur (see Fig. 3a). This is seen continuously at closely spaced intervals along most of the length of the small intestine in the postprandial state.

Power Propulsion Program

Strong, long-lasting, ring-like contractions of the circular muscle coat, which travel long distances (measured in meters) along the small and large intestines, define power propulsion. The contractions, when recorded by force transducers attached to the serosa or placed inside the intestine, reflect formation of the propulsive segment of the hard-wired propulsive reflex in Fig. 2 and were called “giant migrating contractions” by Sushil K. Sarna because they are considerably stronger than the phasic contractions of the circular muscle coat that appear in the activity front of the MMC and in the postprandial motility pattern [12]. Giant migrating contractions have long durations lasting 18–20 s. John R. Mathias studied the electrical equivalents of the giant migrating contractions and called them “migrating action potential complexes” [13]. The giant migrating contractions are a component of efficient propulsive motility that rapidly empties the lumen as they travel at ~ 1 cm/s over protracted lengths of intestine.

Power propulsion differs from propulsive motility in the activity front of the MMC and in the short-segment mixing seen when the postprandial program is “running.” The contractions of the circular muscle, in the propulsive segment, are much stronger and propagation takes place over much longer stretches of intestine. The circular muscle contractions are not time-locked to the electrical slow waves, as is the case during “running” of the postprandial motor program. Release of acetylcholine and/or substance P at neuromuscular junctions, from excitatory musculo-motor neurons, accounts for the large amplitude of the circular muscle contractions.

Aboral Power Propulsion Program

Power propulsive transport of luminal content is bidirectional, along either an oral or aboral trajectory. “Running” of the aboral power propulsive program quickly transports the luminal contents of the distal small intestine or the contents of the colon toward the anus (see Fig. 3e). Noxious mucosal stimulation is one of the triggers that “calls-up” this motor program and closes any other program that might be “running.” Abdominal cramping pain and sometimes fecal urgency and diarrhea are associated with this motor behavior [14]. Exposure of the mucosa to irritants, parasitic infections, enterotoxins from pathogenic bacteria, allergic reactions to food, or exposure to ionizing radiation can start the aboral power propulsive program. These characteristics suggest that this program is a defensive adaptation for rapid clearance of threats from the intestinal lumen [14]. This kind of motor program also underlies mass movement of intraluminal content in normal states, particularly in the distal ileum and during defecation.

The aboral power propulsive program is part of a more extensive “defensive response” that is called-up by exposure to chemical neuromodulators released during degranulation of enteric mast cells and other inflammatory/immune cells [15, 16]. Reconfiguration of the ENS networks by an overlay of these neuromodulators integrates output of a secretomotor program with motility of the power propulsive program in an interlocked sequence. The secretomotor program component first evokes exaggerated mucosal secretion of electrolytes and H_2O that “flushes” threats (e.g., microorganisms, allergens, noxious substances) into suspension in copious volumes in the lumen. The secretory response is followed-up closely by “running” of aboral power propulsion. This rapidly propels the volume of luminal liquid toward the anus. Arrival of the large volume rapidly distends the recto-sigmoid region, which triggers the ENS recto-anal reflex and relaxation of the smooth muscle of the internal anal sphincter. Opening of the internal sphincter at this time underlies sensations of fecal urgency and emotional anxiety because the only remaining protection against incontinence is spinally evoked reflex contraction of the puborectalis and external anal sphincter. Cramping abdominal pain, in these cases, accompanies the large amplitude propulsive contractions evoked during power propulsion [14]. Symptoms of acute, explosive watery diarrhea are to be expected at this stage of operation of the defensive program.

The symptoms associated with “running” the defensive program are reminiscent of the hallmarks of several digestive disorders. Cramping abdominal pain, fecal urgency, and acute watery diarrhea are commonly

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Fig. 3 Depiction of the enteric nervous system viewed, heuristically, as a “mini-brain” with a library of applications, like digitally programmed “apps,” for multiple patterns of small or large intestinal behavior. **a** A specific app determines motor behavior in the postprandial state. **b** Running of a physiologic ileus app programs for a quiescent bowel. **c** Another app establishes the pattern of intestinal motility, called the migrating motor complex, which characterizes the fasting state. **d** A haustral app programs for the formation of haustral pouches in the colon. **e** The power propulsive

app is a protective response to harmful agents in the lower small bowel and colon. It is programmed motor behavior, reflected as strong, long-lasting contractions of the circular muscle that propagate for extended distances in the aboral direction. **f** Propulsive motility in the upper one-third of the small intestine during emesis reflects output of another of the app in the library. During emesis, power propulsion in the upper one-third of the small intestine is reversed for rapid movement of the luminal contents toward the stomach

recognized symptoms associated with: (1) postprandial irritable bowel syndrome, (2) enteritis, both infectious and radiation-induced, (3) ulcerative colitis, (4) Crohn’s disease, (5) microscopic colitis, (6) collagenous colitis, and (7) psychogenic stress.

Emesis Program

The propulsive motility, occurring in the upper one-third of the small intestine during emesis, reflects “running” of another one of the programs in the ENS library. During emesis, the direction of power propulsion in the upper small intestine is reversed for rapid anti-directional transport of the luminal contents toward the stomach (see Fig. 3f). The program is called-up from the library either by commands from the brain or by local sensory detection

of threatening substances in the lumen. As is the case for aboral power propulsion in the small and large intestine, the adaptive significance of the emesis program is defensive removal of threats from inside the lumen of the upper small bowel.

Physiologic Ileus Program

Ileus is defined, historically, as mechanical, dynamic or adynamic obstruction of the bowel [17]. Adynamic ileus is pathologic obstruction of the bowel due to failure of the smooth muscle to contract; dynamic ileus is pathologic intestinal obstruction due to spastic contraction (i.e., failure to relax) of the circular muscle coat in a segment of bowel. In view of the canonically defined characteristics of pathologic ileus, I invented “physiologic ileus” as a

beneficial term for referencing absence of motility in normal small or large intestine [18]. Physiologic ileus is therefore a functional state of the bowel during which the ENS actively inhibits contraction in the autogenic smooth muscle.

Quiescence of the intestinal circular muscle in physiologic ileus reflects the activity of a program in the ENS library, which, when “running,” consists of sustained firing of the inhibitory musculomotor neurons, which innervate the musculature. When active, inhibitory musculomotor neurons release inhibitory neurotransmitters that suppress responsiveness of the circular muscle to electrical slow waves, generated by networks of interstitial cells of Cajal. The physiologic ileus program is active for various lengths of time determined by the digestive state. For example, when the interdigestive program is “running” in the small intestine, physiologic ileus is ongoing in the regions of intestine above and below the propagating activity front of the MMC and is apparent above and below “giant” circular muscle contractions on records of power propulsion (see Fig. 3b–f).

Apps

With “tongue-in-cheek,” in 2015, I started referring to the behavioral programs in the ENS library, as “apps.” This originated from the comment of a “bright” medical student who opined in class that the ENS motility programs being discussed were analogous to collections of mobile “apps” (software applications) commonly stored in the memories of modern smart phones, tablet computers, and other comparable digital devices. I appreciate the student’s apps analogy because it serves to bring to the fore the complexities of the ENS and how little we currently understand about how it works. We do not know, for example, the specifics of hierarchical mechanisms that come into play during rapid conversion from the “postprandial app” to “emetic power propulsion” evoked by administration of the emetic apomorphine, as described in Hans J. Ehrlein’s fluoroscopic studies of movements of intraluminal barium in dogs [19]. Knowledge of this nature is lacking in many areas of ENS neurophysiology beyond this example, especially in applications to understanding of ENS neuropathies.

Enteric Neuropathy

Drug-Induced Neuropathy

Neuropathic action of narcotics in the ENS is expressed as constipation at the whole animal level of organization [20,

21]. Opiates and opioids induce non-propulsive intestinal motility and suppress mucosal secretion of H₂O and electrolytes, each of which reflects suppression of excitability in ENS motor neurons [22–24]. Morphine, the classical opiate, slows intestinal transit by elevating contractile tone in the intestinal circular muscle coat in animal models and humans. Delayed transit is linked with rhythmic contractions at electrical slow wave frequency and with infrequent high-amplitude, non-propulsive phasic contractions [25, 26].

Contractile behavior, associated with opiates or opioids, reflects depressed excitability of inhibitory musculomotor neurons in the ENS and release of the autogenic circular muscle coat from inhibitory neural suppression [23, 27]. Moreover, presynaptic inhibitory action of morphine suppresses release of acetylcholine at the thousands of nicotinic synapses in the ENS microcircuitry [22, 28]. Suppression of nicotinic neurotransmission by morphine disrupts propulsive motility due to interruption of synaptic integration within the ENS’s library of programs. Coordination of contractile behavior of the muscle coats into organized motor patterns requires functional synaptic connectivity at lower levels of the hierarchical order to effectively “run” the programs in the library positioned at the highest tiers of organization.

Narcotic-induced slowing of intestinal transit was assumed to prolong time for absorption of H₂O and electrolytes and therefore became a logical explanation for the constipating symptoms of opiates and opioids. Nevertheless, progress in understanding the mechanisms of ENS control of mucosal secretion and the function of secretomotor neurons in the submucosal division of the ENS has rendered this explanation incomplete. Firing of secretomotor neurons evokes mucosal secretion of H₂O, electrolytes and mucus. Opiates and opioids silence secretomotor neurons by hyperpolarizing their membrane potential below firing threshold. Suppression of secretomotor firing reduces secretion and results in lowered liquidity of the small and large intestinal contents. In the colon, drier-harder stools are the result of secretomotor neuronal suppression.

Inhibitory actions of opiates and synthetic opioids on secretomotor neurons and cholinergic neurotransmission in the ENS account for much of their constipating action. Nevertheless, stimulation of sympathetic noradrenergic input to the ENS underlies some of the constipating action [29–31]. Sympathetic postganglionic fibers project into ENS ganglia where they release norepinephrine at their synapses with ENS neurons. Norepinephrine suppresses fast and slow excitatory synaptic transmission in the neural microcircuits thereby suppressing motility associated with the various programs in the ENS library. At the same time, norepinephrine hyperpolarizes the membrane potential and

suppresses firing in secretomotor neurons in the submucosal plexus. Stimulation of noradrenergic and opioid receptors in the ENS can thereby cause constipation by synergistic suppression of neurogenic mucosal secretion and intestinal motility.

Musculomotor Neuropathy

Musculomotor neurons are the final common output pathways for transmission of control signals from the ENS neural networks to the smooth musculature of the gut. The pool of musculomotor neurons in the ENS is comprised of excitatory and inhibitory motor neurons. ENS excitatory musculomotor neurons release neurotransmitters that evoke contraction of the musculature. Acetylcholine and substance P are the main neurotransmitters that stimulate the smooth musculature to contract. ENS inhibitory musculomotor neurons release neurotransmitters that suppress contractile activity of the smooth musculature. Vasoactive intestinal polypeptide, nitric oxide and ATP are among the neurotransmitters identified as inhibitory neurotransmitters at neuromuscular junctions in smooth musculature throughout the digestive tract.

Inhibitory Musculomotor Neurons

ENS inhibitory musculomotor neurons are a central focus in consideration of ENS neuropathy because neuropathic loss is manifest as multiple forms of disordered small and large intestinal motility, as well as achalasia in smooth muscle sphincters. Disordered motor behavior associated with loss of inhibitory musculomotor function reflects the specialized physiology of the smooth musculature. The circular muscle coat of the small and large intestines, which is responsible for generating the contractile forces for propulsion, is best described as a self-excitable electrical syncytium that incorporates a system of pacemaker cells known as interstitial cells of Cajal (ICCs). In an electrical syncytium, action potentials and pacemaker potentials spread by way of gap junctions from muscle fiber to muscle fiber in three dimensions throughout the tissue, in much the same way as in cardiac muscle. The action potentials trigger contractions as they spread through the musculature. The ICCs are a non-neural pacemaker system of electrical slow waves that are electrically coupled to the musculature and account for most of the self-excitable characteristics of the muscle [32, 33]. The electrical slow waves, generated by the ICCs, are an extrinsic factor to which the circular muscle coat responds. Consideration of these physiologic characteristics of the musculature raises the important questions as to why the circular muscle fails to respond with action potentials and

contractions to each and every pacemaker cycle and why action potentials and associated contractions do not spread in the syncytium throughout the entirety of the ~9 m-length of human small intestine whenever they occur at any point along the bowel. The answer is that the circular musculature in a segment of intestine can only respond to invading electrical slow waves from ICCs when the inhibitory motor neurons in the ENS of that segment are inactivated by input from the control circuits formed by synaptically connected interneurons. Likewise, action potentials and associated contractions can propagate only into regions of musculature where the inhibitory motor neurons are inactivated. Consequently, activity of inhibitory musculomotor neurons determines when the omnipresent slow waves initiate a contraction, as well as the distance and direction of propagation within the syncytium once the contraction has begun.

Because some of the inhibitory musculomotor neurons to the circular musculature fire continuously, action potentials and contractions of the muscle are permitted only when the inhibitory neurons are inactivated by input from interneuronal control circuits [34, 35]. The firing behavior of inhibitory musculomotor neurons to smooth muscle sphincters (e.g., lower esophageal and internal anal sphincters) is opposite to that of the intestinal circular muscle coat. Inhibitory musculomotor neurons, which innervate the sphincters, are normally silent and are switched to firing mode with timing appropriate for opening of the sphincter in coordination with physiologic events in the musculature on either side of the sphincter. When inhibitory musculomotor neurons fire, they release inhibitory neurotransmitters that relax ongoing muscle contraction in the sphincteric muscle and prevent excitation–contraction in the musculature on either side of the sphincter from spreading into and closing the sphincter.

In the non-sphincteric circular muscle coat, the activity state of the inhibitory innervation determines the length of a contracting segment by controlling the distance of spread of action potentials within the three-dimensional electrical geometry of the smooth muscle syncytium. Contraction can occur only in intestinal segments in which ongoing inhibition is inactivated while adjacent segments with continuous inhibitory input cannot contract. Relaxed boundaries on either side of such a contracted segment reflect the transition zone from inactivity to active firing of inhibitory musculomotor neurons. The directional sequence in which the inhibitory musculomotor neurons are inactivated establishes the direction of propagation of the contraction. Normally, inhibition is progressively inactivated in the aboral direction, resulting in contractile activity that propagates in the aboral direction. During emesis, the inhibitory motor neurons must be inactivated in a reverse sequence to account for small intestinal propulsion that

Fig. 4 Image of anti-ENS neuronal antibodies in the serum from a patient with irritable bowel syndrome bound to enteric neurons in the myenteric and submucosal plexuses of guinea pig small intestine.

Ganglia were immunostained with fluorescently labeled anti-human immunoglobulin-G following incubation with the patient's serum. Antibodies in the serum reacted with virtually all ganglion cells

travels toward the stomach. In general, any treatment or condition that ablates intrinsic inhibitory musculomotor neurons results in paroxysmal contractile behavior of the intestinal circular muscle coat.

Many conditions associated with ablation of enteric inhibitory musculomotor neurons are associated with conversion from a hypoactive contractile condition of the circular musculature to a hyperactive contractile state. Observations of hyperactive disorganized motility, commonly made by manometric recording methods in vivo, reinforce the evidence that a subset of the pool of inhibitory musculomotor neurons is tonically active and that drug-induced blockade or ablation of these neurons releases the circular muscle from the inhibitory influence [34–36]. The behavior of the muscle in these cases is tonic contracture and recurring disorganized phasic contractile activity reminiscent of fibrillation in the myocardium.

Disinhibitory ENS Motor Neuropathy

The physiology of ENS inhibitory neuromuscular control in the intestine predicts that spasticity and “achalasia” (i.e., failure to relax) will accompany any condition in which inhibitory musculomotor neurons are destroyed or congenitally absent, as in Hirschsprung's disease. In the absence of ENS inhibitory neuromuscular control, the self-excitable smooth muscle contracts continuously and behaves as an obstruction. This happens because the musculature is liberated to respond to the ICC pacemakers (i.e., electrical slow waves) with contractions that propagate without amplitude, distance or directional control. Contractions spreading in the uncontrolled syncytium collide randomly resulting in chaotic-ineffective motility in the affected intestinal segment.

Loss or malfunction of inhibitory musculomotor neurons is the pathophysiologic starting point for disinhibitory motor disease, which includes multiple forms of chronic intestinal pseudoobstruction and sphincteric achalasia. Neuropathic ENS degeneration of autoimmune origin includes loss of inhibitory musculomotor neurons and is a progressive disease that in its early stages may manifest as symptoms that might be interpreted as irritable bowel syndrome (IBS).

Autoimmune Neuropathy

Collaborating investigators, working at the University of North Carolina (UNC) and The Ohio State University (OSU), tested a hypothesis that symptoms in patients with a diagnosis of IBS reflect disordered neurophysiologic function in the ENS. The UNC-OSU group evaluated the prevalence of anti-ENS autoimmune antibodies and the antigens recognized by the antibodies in patients diagnosed with idiopathic IBS. The approach was based on one used productively for evaluation of anti-enteric neuronal antibodies in patients with gastrointestinal disorders associated with a paraneoplastic syndrome [37–42].

The patients in the UNC-OSU study were categorized as diarrhea predominant (IBS-D), constipation predominant (IBS-C), or alternating/mixed (IBS-A/M) according to *Rome II* symptom-based criteria for a diagnosis of IBS [43, 44]. Sera from 84 % of IBS patients contained antibodies that bound and labeled ENS neurons in an immunostain assay (Fig. 4). All neurons were labeled; no other kinds of cells in the ENS were labeled. Analysis, with sensitivity for 8000 human proteins associated with the immunoglobulin lambda locus, detected antibody reactivity for only three autoantigens in serum samples containing anti-enteric

neuronal antibodies. No antibody reactivity for the same autoantigens was found in serum samples that did not contain the antibodies in immunostain assays. Antibody titers for a nondescript macromolecular complex containing both protein and RNA molecules (i.e., ribonucleoprotein complex) and small ribonuclear polypeptide A were present in high titers in the same sera that contained anti-enteric neuronal antibodies in the immunostain assays. Moderate levels of anti-Ro 52000 antibody were present also in antibody-containing IBS sera and not in sera devoid of antibodies in immunostain assays. The correlation between anti-enteric neuronal antibodies and IBS symptoms was significant. Results of the study supported the hypothesis that symptoms in a subset of IBS patients might be a reflection of enteric neuronal damage or loss, caused by circulating anti-enteric autoimmune antibodies [45].

The UNC-OSU study suggests the possibility for development of a reliable laboratory-based diagnosis useful for explaining to IBS patients the underlying pathophysiology of the symptoms. This can be done with confidence because there is no question that a normally functioning ENS is essential for normal gut function and freedom from gut-related symptoms. The presence of anti-enteric autoantibodies in a patient's serum is a warning that enteric neurons are being destroyed by induction of apoptosis, as is the case for ENS destruction in a paraneoplastic syndrome [41]. Included in the population of destroyed neurons are inhibitory musculomotor neurons that innervate the lower esophageal sphincter. Dysphagia and sphincteric achalasia inevitably occur, as was first shown by Verne et al. [46] for patients with achalasia and anti-enteric neuronal antibodies in their sera.

In cases where ENS autoimmune destruction progresses in the small bowel, the patient is expected to experience IBS-like symptoms correlated with inflammation-related destruction of the ENS, as is found in laparoscopically obtained full-thickness biopsy specimens from patients with IBS [47, 48]. The evidence suggests that the autoimmune attack will eventually culminate in pseudoobstruction, because a functional ENS is essential for propulsive motility.

Laparoscopically obtained, full-thickness intestinal biopsy is an extreme approach for obtaining a diagnosis; nevertheless, it is the only means at the present time of obtaining a definitive diagnosis of autoimmune ENS neuropathy. If the UNC-OSU hypothesis continues to be supported by further testing, then evaluation of the patient's serum for anti-enteric antibodies could prove advantageous for obtaining a diagnosis that would relieve significant anxiety about cause of symptoms in IBS patients without resorting to laparoscopically obtained, full-thickness biopsies. This would be true especially if an

enzyme-linked assay became possible based on the three antigens thus far targets of an immune attack.

Intestinal Pseudoobstruction

Canonic intestinal pseudoobstruction is a pathophysiologic condition in which the symptoms resemble those of a mechanical obstruction that impedes forward propulsion, but without the presence of a mechanical obstruction. Chronic intestinal pseudoobstruction can be myopathic or neuropathic. The neuropathic form of chronic intestinal pseudoobstruction is a form of disinhibitory musculomotor disease linked with neuropathic degeneration in the ENS. Failure of propulsive motility in the affected length of bowel reflects loss of the ENS microcircuits that program and control the repertoire of motility patterns required for the necessary functions of the affected region of bowel.

Pseudoobstruction occurs, in part, because contractile behavior of the circular muscle is hyperactive and uncontrolled in the affected regions. Hyperactivity, when observed with manometric methods or wireless motility capsules, is a diagnostic sign of the neuropathic form of chronic small bowel pseudoobstruction. The hyperactive and disorganized contractile behavior reflects the absence of inhibitory nervous control of the autogenic musculature that is released from the braking action imposed by ENS inhibitory musculomotor neurons. Chronic pseudoobstruction is therefore symptomatic of the advanced stage of a progressive enteric neuropathy and underscores the fact that propulsive motility is impossible in the absence of the ENS. Retrospective review of patient histories suggests that some IBS-like symptoms can be an expression of early stages of ENS neuropathy [47–50].

Non-inflammatory Degenerative Neuropathy

Degenerative non-inflammatory and inflammatory ENS neuropathies are inherited motor disorders that culminate in chronic pseudoobstruction. The mode of inheritance may be autosomal recessive or dominant. In the autosomal recessive type, the neuropathology includes marked reduction in the number of neurons in the myenteric and submucosal plexuses and round, eosinophilic intranuclear inclusions in about one-third of the residual neurons [51]. The inclusions are proteinaceous filament-forming material reminiscent of deposits in Alzheimer's disease [52, 53]. They are not viral particles, as found in ENS neurons of individuals harboring latent varicella-zoster virus infection [54, 55]. The autosomal dominant form of non-inflammatory ENS neuropathic pseudoobstruction is uncommon; members of two families are described by Roy et al. and Schuffler et al. [56, 57].

Inflammatory Degenerative Neuropathy

Inflammatory ENS neuropathies are distinguished by a dense inflammatory infiltrate confined to ENS ganglia. Paraneoplastic syndrome, Chagas disease, and idiopathic degenerative disease are recognizable forms of pseudoobstruction associated with inflammatory neuropathies.

Paraneoplastic syndrome is a form of pseudoobstruction in which commonality of antigens between small cell carcinoma, in the lungs, and ENS neurons leads to an autoimmune attack, which results in ENS neuronal death [37]. Patients with symptoms of pseudoobstruction and small cell carcinoma have immunoglobulin-G autoantibodies that bind to and kill their ENS neurons [58]. These autoantibodies react with a variety of molecules expressed on the nuclear membrane of neurons, including Hu and Ri proteins, and with cytoplasmic antigens (Yo protein) [39, 59].

Immunostaining with sera from patients with a paraneoplastic syndrome shows a characteristic staining pattern in ENS neurons [39]. Immunostaining that shows binding of anti-enteric neuronal antibodies to ENS neurons is a specific diagnosis for paraneoplastic syndrome, as well as for IBS [45]. Induction of apoptosis is a primary mechanism by which autoimmune antibodies destroy ENS neurons in the paraneoplastic syndrome, chronic pseudoobstruction, and IBS [41].

Association of enteric neuronal loss and symptoms of pseudoobstruction in Chagas disease, like paraneoplastic syndrome, reflects autoimmune attack on the neurons with accompanying symptoms that mimic sphincteric achalasia. Achalasia in smooth muscle sphincters (e.g., lower esophageal sphincter) reflects absence of ENS inhibitory musculomotor neurons that normally innervate the sphincter [46]. *Trypanosoma cruzi*, the blood-borne parasite that causes Chagas disease, has antigenic epitopes that have close identity to antigens expressed by ENS neurons [60]. Antigenic commonality between the parasite and ENS neurons activates the immune system to attack the ENS coincident with an attack on the parasite.

The idiopathic form of inflammatory degenerative ENS neuropathy is unrelated to neoplasms, infectious conditions, or other known diseases [61–66]. On the other hand, the varicella-zoster virus (i.e., *chickenpox*) establishes latency in ENS neurons and can reactivate to lyse and kill the neurons within 48 h of infection [54, 55, 67–69].

Anti-enteric neuronal antibodies in serum samples obtained from IBS patients react with only three antigens expressed by ENS neurons, each of which is a ribonuclear protein. The three antigens are: (1) a nondescript ribonucleoprotein (RNP-complex), (2) small nuclear ribonuclear polypeptide A, and (3) Ro-5200-kD [45]. Each antigen is a protein in the neuron's nucleus that binds RNA. This

implies that expression in serum of autoantibodies, which recognize the three kinds of antigens, may be a diagnostic marker for IBS and other functional gastrointestinal disorders. RNP-complex, the first of the three antigens, is involved in RNA processing and transport. TAR DNA-binding protein (TDP-43), the survival motor neuron protein (SMN), and Fragile-X mental retardation protein (FMRP) are three ubiquitous proteins, in the manner of RNP-complex, that are associated with defects in localized parts of the central nervous system [70]. The second antigen, small nuclear ribonuclear polypeptide A, also known as U-1A, is a 70-kD peptide recognized by anti-ribonuclear protein autoantibodies associated with mixed connective tissue diseases (i.e., arthralgias, Raynaud's phenomenon, arthritis, myositis, pulmonary fibrosis, and sclerodactyly [71, 72]. Autoantibodies that recognize the third antigen, Ro-5200-kD, were first reported in the serum of patients with a diagnosis of Sjögren's syndrome [73]. Aside from Sjögren's syndrome, an Anti-Ro5200-kD, reactivity is found in systemic lupus erythematosus, dermatomyositis, and systemic sclerosis [74, 75]. The identity of the three antigens and their localization in the protected environment of a neuronal nucleus prompt the question of how the immune system ever encounters them during the lifetime of an individual. An intriguing hypothetical answer is that activation of a latent viral infection leads to lysis and thereby exposure of the nuclear antigens to detection by the immune system, as is the case for varicella-zoster virus [54, 55, 67–69].

De Giorgio et al. and Smith et al. described two small groups of patients who had early complaints of IBS-like symptoms that progressively worsened over an extended period of time and who eventually received a diagnosis of chronic intestinal pseudoobstruction due to idiopathic degenerative inflammatory neuropathy [65, 76]. Full-thickness biopsy specimens, taken during exploratory laparotomy, showed that each patient had inflammatory infiltrates localized to the myenteric plexus. Immunostaining found that the patients' sera contained antibodies that were reactive with ENS neurons [65].

Stem Cells

I routinely quote anecdotal comments from gastroenterologic colleagues about individual patients they have treated for IBS symptoms, who after several years present with signs of a mechanical intestinal obstruction. Exploratory laparotomy then confirms a diagnosis of intestinal pseudoobstruction, and full-thickness biopsy specimens show ENS ganglionitis. In these conversations, I am often asked the question: "If the IBS-like symptoms and eventual pseudoobstruction reflect an autoimmune enteric

neuropathy, why do the patients have IBS symptoms for so long before presenting with pseudoobstruction.” The answer may be that omnipresent neuronal stem cells divide and give rise to daughter cells that differentiate into one or the other of the specific classifications of ENS neurons and thereby replace neurons killed by autoimmune attack. Replacement “wiring” of newly minted neurons into their synaptic networks may repair the networks and restore their functionality. Neurogenesis of this nature is known to be ongoing continuously in specific regions of the adult brain where the new neurons become integrated into functional microcircuitry [77, 78]. Evidence that a comparable phenomenon occurs in the ENS has begun to emerge.

Evidence for ENS neurogenesis derived from indwelling stem cell populations is convincing and exciting for the subspecialty of *neurogastroenterology* [79–82]. Takaki et al. directed their research effort to restoration of functional recto-anal inhibitory reflex connections and defecation subsequent to resection and anastomosis in a guinea pig model. They obtained the first evidence that serotonergic action at the 5-hydroxytryptamine 4 (5-HT₄) receptor subtype might be a signal for differentiation of ENS stem cells into functional neurons that become “wired” into a reflex circuit that restored functional colonic motility [80]. Liu and Gershon found that neurogenesis, evoked by serotonergic action at the 5-HT₄ receptor subtype, takes place in dedicated stem cell centers outside enteric ganglia in adult mouse intestine [79]. Following mitotic division, the future ENS neurons began a migratory journey of 4–6 months that terminated inside nearby ganglia. This raises the intriguing possibility that these could be the neurons that Takaki et al. found occupying anastomotic sites after intestinal resection, where they differentiate into functional constituents of the recto-anal reflex circuit [80–82].

Serotonergic 5-HT₄ Receptor

The 5-HT₄ receptor subtype is a pharmacotherapeutic target in functional gastrointestinal disorders (e.g., IBS) for drugs such as tegaserod, mosapride, renzapride and cisapride [83]. Pharmacologic interest in 5-HT₄ receptors stems from specific investigative findings that enteric neurons express the mRNA transcript that encodes the 5-HT₄ receptor and that immunohistochemical methods show ENS neuronal surface expression of the receptor in mice and guinea pigs [84, 85]. Exposure to 5-HT₄ receptor agonists enhances fast excitatory postsynaptic potentials at nicotinic synapses in ENS microcircuits of guinea pigs and mice [84, 86, 87]. Release of neurotransmitters associated with peristaltic propulsion (e.g., acetylcholine, substance P,

vasoactive intestinal peptide, nitric oxide) is initiated by stimulation of 5-HT₄ receptors [88].

Conversion of stem cells to functional ENS neurons now joins the list of 5-HT₄ serotonergic signaling functions in the gut. How this kind of action might have influenced the outcomes of the many clinical trials that documented efficacy for one or the other of the various 5-HT₄ receptor agonists or antagonists is unclear [83].

Evidence for serotonergic stimulation of stem cell conversion to new ENS neurons, in a therapeutically significant manner, is compelling. Transection of the rectum and end-to-end anastomosis in guinea pigs severs the neural connections for the recto-anal reflex seen normally as relaxation of contractile tone in the internal anal sphincter in response to balloon distension applied above the anastomosis. Return of the reflex requires about 8 weeks. Exposure of the anastomotic site to the 5-HT₄ receptor agonist, mosapride, shortens the recovery time significantly. Repair of the reflex circuit is by neurogenesis via stem cell division and differentiation into functional neurons as reflected by elevated bromodeoxyuridine (BrdU) incorporation in DNA, in association with stem cell markers inside the anastomotic site [82].

Nevertheless, involvement of 5-HT₄ receptor stimulation is not the full story because data similar to those obtained with 5-HT₄ receptor agonists have been reported for treatment of the anastomotic site with brain-derived neurotrophic factor in the recto-anal reflex model [81].

Gershon et al. published compelling evidence for 5-HT₄ receptor-mediated transition from stem cells to neurons in the ENS of adult mice [79]. Extended administration of BrdU via osmotic pumps to identify proliferating neurons did not find ENS neurons in mitotic states in adult mouse intestine. However, application of a 5-HT₄ receptor agonist along with BrdU administration produced proliferating cells identified as neuron-like [79]. Mitotic cells, which expressed neuronal markers at the start of BrdU administration, were in clusters outside ganglia. After 24 weeks of BrdU administration, cells expressing the marker appeared as small neurons distributed among other neuronal residents inside ganglia. Based on these observations, Gershon et al. suggested that enteric neurogenesis occurs in vivo, but at rates too low to be detected unless the rate is increased by a stimulus, such as activation of serotonergic 5-HT₄ receptors [79]. The 5-HT₄ hypothesis is supported further by findings that selective 5-HT₄ receptor antagonists suppress the proliferation and migration of emergent neurons into positions inside ganglia [79]. Moreover, the results of Liu and Gershon were essentially the same when their extended BrdU study was done in 5-HT₄ knock-out and wild-type mice. Serotonergic stimulation of neurogenesis occurred in wild-type mice and was absent in the knock-outs.

Epilogue

The heuristic model for the ENS is a canonical independent integrative nervous system. The ENS, like all other independent integrative nervous systems in the animal kingdom, is organized in a tiered hierarchy of structures and functions ranging from the cellular-molecular at the lower tiers to plasticity in programmed synaptic networks (“apps”) at the uppermost tiers. Malfunctions at most any level of the hierarchal organization disrupt normal gut motility, and pathologic symptoms emerge. The gut, without an ENS, has no functional propulsive motility. Autoimmune ablation of the inhibitory motor innervation of the lower esophageal sphincter underlies achalasia, megaesophagus, and disordered swallowing. Congenital absence of the ENS is the hallmark of the constricted terminal segment in Hirschsprung’s disease. Loss of inhibitory motor innervation of the sphincter of Oddi elevates pressures in biliary tract ducts, distends the ducts, and causes acalculous biliary pain. Chronic intestinal pseudoobstruction arises from autoimmune attack in parasitic infection, or small cell lung carcinoma, or on an idiopathic basis. Life is hard for an ENS neuron living in the gut wall environment where it is exposed to multiple destructive insults. Stem cell neurogenesis replaces neurons in damaged microcircuits and restores neural network functionality.

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