Fundamentals of Neurogastroenterology: Basic Science

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The focus of neurogastroenterology in Rome II was the enteric nervous system (ENS). To avoid duplication with Rome II, only advances in ENS neurobiology after Rome II are reviewed together with stronger emphasis on interactions of the brain, spinal cord, and the gut in terms of relevance for abdominal pain and disordered gastrointestinal function. A committee with expertise in selective aspects of neurogastroenterology was invited to evaluate the literature and provide a consensus overview of the *Fundamentals of Neurogastroenterology* textbook as they relate to functional gastrointestinal disorders (FGIDs). This review is an abbreviated version of a fuller account that appears in the forthcoming book, *Rome III*. This report reviews current basic science understanding of visceral sensation and its modulation by inflammation and stress and advances in the neurophysiology of the ENS. Many of the concepts are derived from animal studies in which the physiologic mechanisms underlying visceral sensitivity and neural control of motility, secretion, and blood flow are examined. Impact of inflammation and stress in experimental models relative to FGIDs is reviewed as is human brain imaging, which provides a means for translating basic science to understanding FGID symptoms. Investigative evidence and emerging concepts implicate dysfunction in the nervous system as a significant factor underlying patient symptoms in FGIDs. Continued focus on neurogastroenterologic factors that underlie the development of symptoms will lead to mechanistic understanding that is expected to directly benefit the large contingent of patients and care-givers who deal with FGIDs.

Neurogastroenterology is an emerging area of scientific and clinical subspecialization that was introduced in the early 1990s. Neurogastroenterology encompasses basic and clinical research dealing with function and dysfunction of the gastrointestinal (GI) tract and its neural innervation. In Rome II, attention was focused on the enteric nervous system (ENS) and neuroeffector mechanisms as they relate to functional gastrointestinal disorders (FGIDs).[1,2] Also relevant are the central nervous system (CNS) mechanisms that process and interpret the incoming sensory information that gives rise to visceral pain and influence the autonomic sympathetic and parasympathetic outflows that, together with the ENS, control and coordinate digestive functions. Clinical gastroenterology translates basic discovery into the diagnosis and treatment of FGIDs and includes the impact of inflammation and psychological state on brain-gut interactions. This report continues the "fundamentals" with a primary focus on interactions of the brain, spinal cord, ENS, and gut and the relevance for abdominal pain and disordered GI function.

Visceral Pain and Sensation

GI afferents mediate reflexes that control motility, secretion, and blood flow and also modulate immune responses.[3] Moreover, sensory information reaching the CNS gives rise to both painful and nonpainful sensation and influences feeding and illness behavior. Heightened visceral sensitivity is a hallmark of FGIDs. Whether the hypersensitivity reflects transmission of aberrant sensory signals to the brain, normal signals that are interpreted inappropriately by the brain, or a combination of both remains an unresolved question.

Peripheral Sensory Physiology

Vagal and spinal afferent nerve fibers transmit sensory information from the GI tract to the CNS. Vagal afferents have cell bodies in nodose ganglia and enter the

Abbreviations used in this paper: AT-II, angiotensin II; CNS, central nervous system; CRF, corticotropin-releasing factor; EGC, enteric glial cell; ENS, enteric nervous system; EPSP, excitatory postsynaptic potential; FGID, functional gastrointestinal disorder; GI, gastrointestinal; 5-HT, serotonin; IBS, irritable bowel syndrome; IL, interleukin; IPSP, inhibitory postsynaptic potential; IR, immunoreactivity; MMC, migrating motor complex; PAR, protease-activated receptor; TNBS, trinitrobenzene sulfonic acid.

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brainstem. Cell bodies of spinal afferents are located in dorsal root ganglia and project to the dorsal horn of the spinal cord and the dorsal column nuclei. Spinal afferents are broadly subdivided into splanchnic and pelvic afferents that follow the paths of sympathetic and parasympathetic efferents to the gut wall. Somatic afferents, which innervate the striated musculature of the pelvic floor, project to the sacral spinal cord via the pudendal nerve.

Peripheral endings of vagal and spinal sensory neurons terminate within the musculature, mucosal epithelium, and ganglia of the ENS.[3] Spinal afferents also terminate in the serosa and mesenteric attachments and form a dense network around mesenteric blood vessels and their intramural tributaries. Vagal afferent endings in the mucosa are in close association with the lamina propria adjacent to the mucosal epithelium, where they directly monitor the chemical nature of luminal contents either directly following passage across the epithelium or indirectly via paracrine input from enteroendocrine cells in the epithelium.[3] Luminal nutrients, for example, cross the epithelium by various transport mechanisms to reach the afferent nerve terminals in the lamina propria. In addition, luminal nutrients act before absorption to cause the release of messenger molecules (eg, cholecystokinin and serotonin [5-HT]) from enteroendocrine cells in the mucosa. These molecules in turn act on afferent terminals that lie in close proximity in the lamina propria.[4,5]

Vagal afferent endings in the GI wall are classified as either intramuscular arrays or intraganglionic laminar endings. Intramuscular arrays are distributed within the muscle sheets running parallel to the long axes of the muscle fibers,[6] where they appear to make direct contact with the muscle fibers and also form appositions with intramuscular interstitial cells of Cajal. Intraganglionic laminar endings are basket-like structures associated with myenteric ganglia in the ENS. The location of intraganglionic laminar endings between the circular and longitudinal muscle layers exposes them to the shearing forces generated during muscle stretch or contraction and determines their function as low-threshold mechanoreceptors.[7] Intraganglionic laminar endings are also present in the pelvic supply to the rectal musculature.[8] Their location in regions from which graded sensory experiences can arise in response to investigator-applied stimuli (eg, balloon distention) leads to a suggestion that these endings may signal nonpainful sensations of fullness.

Spinal afferents have multiple receptive fields extending over relatively wide areas of bowel.[3] Afferent endings in the serosa and mesenteric attachments respond to distortion of the viscera during distention and contraction. Other endings detect changes in the submucosal chemical milieu following injury, ischemia, or infection and may play a role in generating hypersensitivity to distention and muscle contraction.[5]

Intramural spinal afferent fibers have collateral branches that innervate blood vessels and enteric ganglia. These contain and release neurotransmitters during local axon reflexes that influence GI blood flow, motility, and secretory reflexes.[9] Spinal afferents en route to the spinal cord also give off collaterals that innervate prevertebral sympathetic ganglia.[10] The same sensory information is thereby transmitted to information-processing circuits in the spinal cord, ENS, and prevertebral ganglia. Calcitonin gene-related peptide and substance P are important neurotransmitters in this sensory pathway, and both of these peptides are implicated in the induction of neurogenic inflammation.[11]

Sensory transduction ultimately depends on the modulation of ion channels and/or receptors on the sensory nerve terminal.[3] Mechanosensitivity may arise indirectly following the release of chemical mediators such as adenosine triphosphate (ATP), which in turn can act on purinergic receptors present on afferent nerve terminals. Alternatively, there may be direct activation via mechanosensitive ion channels in the afferent nerve terminals.[5] Mechanical deformation of the nerve ending leads to the opening or closing of the ion channels, which depolarizes the terminal to threshold for action potential firing and transmission of the sensory information to the CNS.

Vagal mechanoreceptors generally have low distention thresholds of activation, as indicated by responses to increases in distending pressures of a few millimeters of mercury and maximal firing frequencies occurring within physiologic levels of distention.[3] However, some vagal fibers can convey information about high-intensity mechanical stimulation and may also respond to noxious chemical stimulation.[12] Spinal afferents are classified as low-threshold, high-threshold, or silent mechanoreceptors.[13] Low-threshold afferents respond to physiologic levels of distention and continue to encode excessive levels of distention that evoke pain in humans and pain behavior in animals. High-threshold afferents respond to higher levels of distention that are in the noxious range. Silent nociceptors do not respond at all in the normal intestine but become responsive to distention when the intestine is injured or inflamed.[12] This kind of receptor behavior illustrates how mechanosensitivity is not fixed, either in terms of the threshold for sensory activation or the relationship between stimulus and response. Injury and inflammation decrease the threshold and increase the magnitude of the response for a given stimulus, a phenomenon known as peripheral sensitization.[14] Inflamma-

tory sensitization underlies the perception of a normally innocuous stimulus as being painful and exaggerates the intensity of pain experienced during a painful stimulus (ie, hypersensitivity).

Sensitizing mediators are released by a plethora of cell types, including blood platelets, leukocytes, lymphocytes, macrophages, mast cells, glia, fibroblasts, blood vessels, muscle, epithelial cells, and neurons. Several mediators can be released from a single cell type to act either directly on the sensory nerve terminal or indirectly by stimulating the release of agents from other cells in a series of cascades.

A battery of chemical mediators, including biogenic amines, purines, prostanoids, proteases, and cytokines, act in a promiscuous manner on a range of receptors expressed on any one sensory ending. Three distinct processes are involved in the actions of these substances on visceral afferent nerves. First, by direct activation of receptors coupled to the opening of ion channels present on nerve terminals, the terminals are depolarized and firing of impulses is initiated. The second is by sensitization that develops in the absence of direct stimulation and results in hyperexcitability to both chemical and mechanical modalities. Sensitization may involve postreceptor signal transduction that includes G protein–coupled alterations in second messenger systems that in turn lead to phosphorylation of membrane receptors and ion channels that control excitability of the afferent endings. The third is by genetic changes in the phenotype of mediators, channels, and receptors expressed by the afferent nerve; for example, a change in the ligand-binding characteristics or coupling efficiency of newly expressed receptors might alter the sensitivity of the afferent terminals. Neurotrophins, in particular nerve growth factor and glial-derived neurotropic factor, influence different populations of visceral afferents and play an important role in adaptive responses to nerve injury and inflammation.[15]

Peripheral sensitization can occur rapidly and be short-lived because the changes taking place at the level of the sensory nerve terminal are dependent on release of one or more algesic mediators. However, in the event of sustained tissue injury or inflammatory states, changes in gene expression can occur that prolong peripheral sensitization. These changes include alterations in those genes that determine the amount and pattern of neurotransmitters released from the sensory nerve terminals in the spinal cord and the brain, thereby altering the CNS processing of sensory information.[5] Peripheral sensitization integrated with central sensitization of this nature is undoubtedly a significant factor determining the sensations of abdominal pain and discomfort associated with FGIDs.

Spinal Cord

Visceral afferents constitute only 10% of all afferent inflow into the spinal cord, yet they have widespread termination in laminae I, II, V, and X of the dorsal horn.[16] Input from visceral and somatic sensory fields converges onto the same neurons in the dorsal horn, dorsal column nuclei, and supraspinal centers.[17–20] Viscerovisceral convergence of sensory information onto the same neurons also occurs in the spinal cord. For example, pelvic visceral inputs from colon and rectum, bladder, uterine cervix, and vagina all converge onto the same second-order spinal neurons.[16,17] The low density of visceral nociceptors, the phenomenon of viscerovisceral convergence, and the functional divergence of visceral input within the CNS probably all contribute to the poor localization of visceral pain to a specific bodily region.

Visceral nociceptive information is transmitted centrally via spinothalamic, spinohypothalamic, spinosolitary, spinoreticular, and spinoparabrachial tracts, all in the anterolateral quadrant of the spinal cord. In addition, a recently discovered pathway in the dorsal columns, which involves mainly postsynaptic neurons, is also involved in viscerosensory processing and visceral pain transmission.[18–25] Pain signals in the dorsal columns are then transmitted via the ipsilateral dorsal column nuclei (ie, nucleus gracilis and nucleus cuneatus) to the contralateral ventroposterolateral nucleus of the thalamus. Stimulation of the posterior columns in a patient with severe irritable bowel syndrome (IBS) evokes an immediate increase in the intensity of abdominal pain.[27] The evidence suggests that dorsal column pathways have a major role in visceral nociceptive transmission.

Central Sensitization

Central sensitization is believed to be the mechanism underlying secondary hyperalgesia, which is a phenomenon of increased pain sensitivity in regions distant to the site of injury or inflammation. Secondary hyperalgesia results from altered mechanisms of synaptic transmission in the spinal cord, which leads to a decrease in threshold, increased responsiveness, and an expansion of spinal neuronal receptive fields.[28] Central sensitization might contribute to the visceral hypersensitivity to distention found in patients with IBS. The changes in synaptic transmission persist beyond the period of initial injury or inflammation and can be associated with altered bowel function.[29,30] Glutamate and substance P are the main neurotransmitters released during the spinal processing of visceral pain. Both *N*-methyl-D-aspartate and

non–*N*-methyl-D-aspartate glutamate receptors and neurokinin receptors are implicated in the synaptic mechanisms underlying central sensitization.

Descending Spinal Modulatory Pathways

At the level of the spinal cord, inputs from nonnociceptive and nociceptive afferent pathways interact to modify transmission of nociceptive information to higher brain centers. The brain itself has modulatory systems that affect the conscious perception of incoming sensory stimuli. Spinal visceral nociceptive transmission is subject to modification by descending modulatory influences from supraspinal structures (eg, periaqueductal gray, nucleus raphe magnus, locus ceruleus, nuclei reticularis gigantocellularis, and the ventrobasal complex of the thalamus). Descending modulation is sometimes inhibitory, facilitatory, or both, depending on the context of the visceral stimulus or the intensity of the descending signal.[30] The descending influence from the ventromedial medulla is mediated mainly by pathways traveling in the dorsolateral spinal cord[31] and can be inhibitory or facilitatory based on stimulus intensity. In contrast, descending control from the thalamus is context specific in that it may facilitate or inhibit spinal nociceptive processing depending on the presence or absence of central sensitization.[31]

Serotonergic, noradrenergic, and, to a lesser extent, dopaminergic projections are major components of descending modulatory pathways. Exaggeration of descending facilitative signals from the brain may partly explain the visceral hypersensitivity that is found in a subset of patients with IBS.[27]

Representation of Sensation in the Brain

Conscious experience of sensation is a multifaceted process that involves a complex interaction between sensory-discriminative, affective, and cognitive dimensions. Functional brain imaging techniques make it possible to study the complex interaction between a number of cortical and subcortical areas involved in sensory experience and may in the future help determine whether sensory dysfunction in patients with FGIDs is due to disordered sensory detection and transmission in the periphery (eg, tissue injury or inflammation), aberrant processing of sensory information in the brain, or a combination of these peripheral and central factors.

Viscerocortical Pain Matrix

The functional brain imaging techniques of functional magnetic resonance imaging and positron emission tomography, both of which rely on measurements of blood flow in cortical and subcortical areas where increased metabolic activity occurs in response to sensory experience, are used to identify a network of brain areas that process GI sensation.[32–37] Unlike somatic sensation, which has a strong homuncular representation in the primary somatosensory cortex, visceral sensation is primarily represented in the secondary somatosensory cortex. Representations in the primary somatosensory cortex are vague and diffuse, which might account for visceral sensation being poorly localized in comparison with somatic sensation. Visceral sensation is also represented in paralimbic and limbic structures (eg, anterior insular cortex, amygdala, anterior and posterior cingulate cortex) and prefrontal and orbitofrontal cortices. These are the areas that process the affective and cognitive components of visceral sensation. Gender differences in cortical representation of rectal sensation occur in healthy volunteers. While activation in the sensory-motor and parietooccipital areas is common in men and women, greater activation in the anterior cingulated/prefrontal cortices was found in women.[37] These gender differences in the processing of sensory input are reminiscent of reports that perceptual responses are exaggerated in female patients with FGIDs.

ENS

The chapter on fundamentals of neurogastroenterology in Rome II provided a review of the neurophysiology of the ENS that was up-to-date at the time.[1,2] Advances since Rome II appear in subsequent reviews[38–42] and are summarized in brief in this section. Attention to the ENS continues to have central importance for neurogastroenterology and FGIDs because the digestive tract does not work without the integrative functions of the ENS. Normal functioning of the neural networks of the ENS is necessary for effective motility, secretion, and blood flow and coordination of these functions into organized patterns of behavior at the level of the integrated organ system. As expected, malfunctions of integrative ENS control of the gut's effector systems are increasingly recognized as underlying factors in GI disorders, especially in the FGIDs, and therefore become a target for drug therapy.[41,43–46]

Enteric Neural Signaling

Fundamental mechanisms for chemically mediated signaling in the ENS are the same as elsewhere in the nervous system and may occur in the form of neurocrine (ie, synaptic transmission), endocrine, or paracrine signals. Transmitters at chemical synapses are released by Ca^{2+}-triggered exocytosis from stores localized in vesicles at axonal terminals or transaxonal varicosities.

Release is triggered by the depolarizing action of action potentials when they arrive at the release site and open voltage-activated Ca^{2+} channels. Once released, enteric neurotransmitters bind to their specific postsynaptic receptors to evoke ionotropic or metabotropic synaptic events. When the receptors are directly coupled to the ionic channel, they are classified as "ionotropic." They are "metabotropic receptors" when their effects to open or close ionic channels are indirectly mediated by guanosine triphosphate binding proteins and the induction of cytoplasmic second messengers (eg, adenosine 3′,5′-cyclic monophosphate, inositol 1,4,5-triphosphate, and diacylglycerol).[47]

The kinds of synaptic events in the ENS are basically the same as in the brain and spinal cord. Fast and slow excitatory postsynaptic potentials (EPSPs) and inhibitory postsynaptic potentials (IPSPs) are principal synaptic events in the ENS. An enteric neuron may express mechanisms for both slow and fast synaptic neurotransmission. Fast synaptic potentials have durations in the millisecond range; slow synaptic potentials last for several seconds, minutes, or longer. Fast synaptic potentials are usually EPSPs. The slow synaptic events may be either EPSPs or IPSPs.

Fast EPSPs

Fast EPSPs were reported for the earliest intracellular studies of myenteric neurons but were found only in S-type neurons in the early work of Hirst et al and Nishi and North.[48,49] Fast EPSPs are rapidly activating depolarizing responses with durations less than 50 milliseconds. Fast EPSPs were later reported to occur in AH- and S-type neurons in both myenteric and submucosal plexuses.[39] Fast EPSPs appear to be the sole mechanism of transmission between vagal efferents and enteric neurons. Most of the fast EPSPs are mediated by acetylcholine acting at nicotinic postsynaptic receptors. The actions of 5-HT at the 5-HT_3 serotonergic receptor subtype and purine nucleotides at P2X purinergic receptors behave much like fast EPSPs, and it is possible that some fast EPSPs are purely serotonergic or purinergic or reflect a summation of purinergic and serotonergic input.[50–55] P2X receptors are potential therapeutic targets.[56] Antagonists at 5-HT_3 receptors have proved effective in treatment of diarrhea-predominant IBS.[57]

Nicotinic Receptors

Different combinations of α and β subunits assemble in different combinations to form nicotinic receptors in general. Functional receptors are formed by 5 subunits. Eight different α subunits (ie, α_{2-9}) and 9 β subunits (ie, β_{2-4}) have been identified. The properties of a specific nicotinic receptor are determined by the kinds of subunits that form the pentameric receptor. The main mediator of nicotinic fast EPSP-like responses in enteric neurons is a receptor composed of α_3 and β_4 subunits.[54]

Purinergic Receptors

P2X receptors are trimeric proteins formed by subunits with 2 transmembrane domains. There are at least 7 subtypes of P2X receptors, ranging from $P2X_1$ to $P2X_7$. Immunohistochemical studies using specific P2X receptor antibodies found at least 3 P2X receptor subunits in the ENS that were identified as $P2X_2$, $P2X_3$, and $P2X_7$.[58–61] The $P2X_2$ receptor is expressed in neurons that express immunoreactivity (IR) for the chemical codes of inhibitory musculomotor neurons, noncholinergic secretomotor neurons, and calbindin-immunoreactive neurons.[60] Loss of the purinergic component of fast EPSPs in $P2X_2$ knockout mice supports the suggestion that the $P2X_2$ receptor is a major player in purinergic fast transmission in enteric neurons.[53] $P2X_3$ subunits form heteromers with $P2X_2$ subunits in aborally projecting inhibitory musculomotor neurons. Calbindin-immunoreactive enteric neurons do not express $P2X_3$ receptor IR.[59,61]

Serotonergic 5-HT_3 Receptors

Responses mediated by 5-HT_3 receptors are found on neurons in both plexuses throughout the GI tract, including the stomach.[62] Neither purinergic nor serotonergic fast EPSPs occur in each and every class of neurons (ie, based on chemical codes) in the guinea pig colon and elsewhere.[55]

Results obtained with patch clamp recording methods show that the nicotinic and 5-HT_3 serotonergic receptors connect directly to nonselective cationic channels. Opening of these channels is responsible for the depolarizing event.[63] Rapid desensitization, within seconds in vitro, is a characteristic of both the nicotinic- and 5-HT_3–operated channels, both of which are ionotropic. Purinergic "fast" depolarizing responses, like serotonergic 5-HT_3 receptor–mediated responses, reflect opening of ligand-gated nonselective cationic channels.[64]

Fast EPSP Rundown

Amplitudes of nicotinic fast EPSPs in the intestine become progressively smaller when they are evoked repetitively by focal electrical stimulation applied to the surface of the ganglion or interganglionic fiber tract in vitro. Decrease in EPSP amplitude occurs at stimulus frequencies as low as 0.1 Hz, and the rate of decline is a direct function of stimulus frequency. Rundown of this nature does not occur at the synapses in the stomach or

gallbladder.[65–67] The rundown phenomenon reflects presynaptic inhibition of acetylcholine release by additional transmitter substances broadly released by the electrical stimulus or by negative feedback involving autoinhibition of acetylcholine release mediated by presynaptic inhibitory muscarinic receptors.[68] Rundown cannot be attributed to postsynaptic changes, because no decrease in the amplitude of the EPSPs occurs during repetitive applications of acetylcholine from microejection pipettes.

Significance of Fast EPSPs

The importance of fast EPSPs emerges from their function in the rapid transfer and transformation of neurally coded information between axons and neuronal cell bodies and axons and dendrites that form the enteric neural networks. Fast EPSPs may or may not depolarize the membrane to its threshold for discharge of an action potential. Summation of multiple inputs increases the probability of reaching firing threshold. Fast EPSPs do not reach threshold when the neuronal membranes are hyperpolarized during slow IPSPs. They are most likely to reach spike threshold when the membranes are depolarized during slow EPSPs or depolarizing action of modulators released in paracrine fashion from nonneuronal cells. This effect of slow EPSPs and of slow EPSP-like paracrine mediators is an example of neuromodulation whereby the input-output relations of a neuron to one input (ie, fast EPSPs) are modified by a second synaptic or other kind of modulatory input.

Slow EPSPs

Slow EPSP and slow EPSP–like excitation evoked by substances released in endocrine or paracrine fashion are major signaling events in the ENS microcircuitry, where many research advancements have been made. Slow EPSPs in enteric neurons with S-type electrophysiologic behavior and uniaxonal morphology differ from neurons with AH-type electrophysiologic behavior and multipolar Dogiel type II morphology.[47] Slow EPSPs in AH-type enteric neurons are associated with slowly activating membrane depolarization, suppression of hyperpolarizing afterpotentials, decreased membrane conductance, and elevated excitability. Postreceptor signal transduction in AH-type neurons involves stimulation of adenylate cyclase and elevation of intraneuronal adenosine 3′,5′-cyclic monophosphate levels. Slow EPSPs in S-type uniaxonal neurons are also slowly activating depolarizing potentials associated with elevated excitability; however, unlike AH-type neurons, membrane conductance either increases or does not change during the EPSP. The postreceptor signal transduction cascade in these neurons involves stimulation of phospholipase C and elevation of intraneuronal Ca^{2+} levels.[47] Exposure to bradykinin, serine proteases, ATP, corticotropin-releasing factor (CRF), or angiotensin II (AT-II) has been reported to mimic slow EPSPs in the ENS since publication of Rome II in 1999 and 2000.[1,2]

Bradykinin. Bradykinin is an established inflammatory mediator derived from proteolytic action on plasma proteins as a result of tissue injury, anoxia, or inflammation. Application of bradykinin evokes slow EPSP-like excitation in AH- and S-type neurons.[69–71] The selective B_2 bradykinin receptor antagonist Hoe 140 but not the selective B_1 receptor antagonist des-arg^{10}-Hoe 140 suppresses responses to bradykinin. Reverse-transcription polymerase chain reaction and Western blot analysis confirm the existence of B_2 receptor messenger RNA and protein in guinea pig myenteric and submucosal plexuses, and binding of fluo-HOE 140 (HOE741) reveals that the neurons are endowed with the B_2 receptors and not the B_1 receptor.[69] The mechanism of excitatory action of bradykinin is somewhat unique in that it acts at the neuronal B_2 receptor to stimulate the neuron to synthesize and release prostaglandin E_2, which feeds back and acts at EP_1 receptors to evoke excitation in the same neuron.[70] Prostaglandin feedback accounts for an earlier finding that bradykinin releases acetylcholine from the myenteric plexus by a prostaglandin-mediated mechanism.[72] Exposure to bradykinin also suppresses the amplitude of both stimulus-evoked slow EPSPs and fast nicotinic EPSPs in the ENS.

Serine proteases and protease-activated receptors. Serine proteases can be released from enteric mast cells to mimic slow EPSPs by stimulating protease-activated receptors (PARs) on the neurons.[73–76] Application of thrombin, trypsin, or mast cell tryptase evokes slow EPSP-like excitatory responses in AH- and S-type enteric neurons. Synthetic activating peptides for PAR-1, PAR-2, and PAR-4 receptors mimic these actions. The depolarizing responses evoked by a majority of PAR-sensitive uniaxonal enteric neurons express IR for nitric oxide synthase.[74] IR for nitric oxide synthase (ie, labeled antibodies raised against nitric oxide label the neurons) suggests that these neurons are descending inhibitory motor neurons to the intestinal circular muscle.

ATP. Secretomotor neurons with vasoactive intestinal peptide IR in the submucosal plexus of guinea pig intestine receive slow EPSP input that is mediated by synaptic release of ATP and its action at $P2Y_1$ receptors expressed by the neurons.[76] MRS2179, a selective $P2Y_1$ purinergic receptor antagonist, blocks both the slow EPSP and mimicry of the EPSP by exogenously applied ATP. The submucosal secretomotor neurons receive their purinergic excitatory input from neighboring neurons in

the same plexus, neurons in the myenteric plexus, and sympathetic postganglionic neurons. The ATP-mediated EPSPs occur coincident with fast nicotinic synaptic potentials evoked by projections from both myenteric neurons and other submucosal neurons and with noradrenergic IPSPs that are evoked by firing of sympathetic fibers that innervated the same neurons.

The $P2Y_1$ receptors on secretomotor neurons are metabotropic receptors that are linked to activation of phospholipase C, synthesis of inositol 1,4,5-triphosphate, and mobilization of Ca^{2+} from intracellular stores. The purinergic neurons that synapse with and release ATP at postsynaptic $P2Y_1$ receptors on submucosal secretomotor neurons themselves express excitatory serotonergic $5\text{-}HT_3$ receptors that respond to exogenously applied 5-HT and might be stimulated by release of 5-HT from mucosal enterochromaffin cells.[76] Purinergic $P2Y_1$ signaling to secretomotor neurons is currently recognized as an important aspect of the functional regulation of intestinal mucosal secretion.[77,78]

CRF. A later section on mechanisms underlying the impact of stress on intestinal motor, secretory, and immune functions presents evidence that stimulation of receptors for CRF in the brain and the ENS of animal models is a factor in stress-induced alteration of GI functions and the exacerbation of FGID symptoms in humans.[79] IR for CRF is expressed in both the myenteric and the submucosal plexuses of all regions of the large and small intestine and the myenteric plexus of the stomach of the guinea pig.[80,81] Most of the CRF-immunoreactive myenteric neurons have uniaxonal morphology; the remainder has Dogiel type II multipolar morphology. CRF-immunoreactive cell bodies in the myenteric plexus of the ileum express IR for choline acetyltransferase, substance P, and nitric oxide synthase. CRF IR never colocalizes with IR for calbindin, calretinin, neuropeptide Y, serotonin, or somatostatin in the myenteric plexus. CRF-immunoreactive cell bodies are more abundant in the submucosal plexus than in the myenteric plexus. All CRF-immunoreactive neurons in submucosal ganglia express vasoactive intestinal peptide IR and are likely to be secretomotor/vasodilator neurons.

Exposure to CRF evokes slowly activating depolarizing responses associated with elevated excitability in both myenteric and submucosal neurons.[80] Histologic analysis of biocytin-filled neurons finds that both uniaxonal neurons with S-type electrophysiologic behavior and neurons with AH-type electrophysiologic behavior and Dogiel II morphology respond to CRF. The CRF-evoked depolarizing responses are suppressed by the CRF_1/CRF_2 receptor antagonist astressin and the selective CRF_1 receptor antagonist NBI 27914 and are unaffected by the selective CRF_2 receptor antagonist antisauvagine-30.[80] Reverse-transcription polymerase chain reaction reveals expression of messenger RNA transcripts for the CRF_1 receptor but not the CRF_2 receptor in both myenteric and submucosal plexuses of guinea pig. IR for the CRF_1 receptor is distributed widely in the myenteric plexus of the stomach and small and large intestine and in the submucosal plexus of the small and large intestine. CRF_1 receptor IR is coexpressed with calbindin, choline acetyltransferase, and substance P in myenteric plexus neurons. In the submucosal plexus, CRF_1 receptor IR is found in neurons that express calbindin, substance P, choline acetyltransferase, or neuropeptide Y. The evidence implicates the CRF_1 receptor as the mediator of the excitatory actions of CRF on neurons in the ENS.

CRF-immunoreactive neurons do not express IR for the CRF_1 receptor. CRF_1 IR is expressed in neuronal neighbors of those with CRF IR, which suggests that secretomotor neurons expressing CRF IR might provide synaptic input to CRF_1 receptors on neighboring cholinergic neurons. A general conclusion is that actions on enteric neurons might underlie the neural mechanisms by which stress-related release of CRF in the periphery alters intestinal propulsive motor function, mucosal secretion, and mucosal barrier function.

Angiotensin. Two enzymes catalyze the conversion of angiotensin I to AT-II, which is the biologically active form in the intestine. One of the enzymes, angiotensin-converting enzyme, is expressed in a variety of tissues and organs, including the brush border of the small intestinal epithelium.[82,83] Mast cell α kinases are a second set of converting enzymes. α-Chymase is the major non–angiotensin-converting enzyme producer of AT-II in humans.[84,85] Release of α-chymase accounts for the appearance of AT-II as one of the main products associated with degranulation of mast cells.[85] Significantly elevated levels of AT-II are found in mucosal biopsy specimens from patients with Crohn's colitis, which suggests that elevated levels might be associated with inflammatory states, including mast cell hyperplasia.[86,87]

The predictable hypertensive action of systemically administered AT-II is well known. Systemic dosing with AT-II evokes vasoconstriction and reduced blood flow in the intestinal mesenteric vasculature in parallel with whole-body hypertension. Elevated vascular resistance and decreased flow in the inferior mesenteric vascular bed leads to ischemic colitis in pigs receiving pathophysiologic doses of AT-II.[88]

AT-II also alters intestinal absorption of Na^+ and H_2O. Low doses of AT-II (eg, $0-60\ ng \cdot kg^{-1} \cdot min^{-1}$) stimulate Na^+ and H_2O absorption.[89,90] Stimulation of absorption is secondary to elevated release of norepineph-

rine from intramural sympathetic nerves in concert with suppression of neuronal reuptake of norepinephrine.[91] Intracerebroventricular administration of AT-II stimulates descending spinal pathways, which activate sympathetic outflow to the bowel.[92]

Inhibitory actions of norepinephrine on enteric secretomotor neurons explain the action of AT-II to suppress mucosal secretion and induce an absorptive state. Secretomotor neurons are well recognized as excitatory motor neurons in the submucosal division of the ENS that innervate the intestinal crypts of Lieberkühn.[93] Firing of secretomotor neurons releases acetylcholine and/or vasoactive intestinal peptide as neurotransmitters at their junctions in the crypts. Secretomotor axons also send collaterals to innervate submucosal arterioles.[94,95] Collateral innervation of the blood vessels links blood flow to secretion by releasing acetylcholine simultaneously at neuroepithelial and neurovascular junctions. Once released, acetylcholine acts at the blood vessels to dilate the vessels and increase blood flow in support of stimulated secretion.

Secretomotor neurons have receptors that receive excitatory and inhibitory synaptic input from neurons in the integrative circuitry of the ENS and from sympathetic postganglionic neurons. Activation of the excitatory receptors on secretomotor neurons stimulates the neurons to fire and release their transmitters at the junctions with the crypts and regional blood vessels. The overall result of secretomotor firing is stimulation of the secretion of H_2O, electrolytes, and mucus from the crypts. Elevated firing of secretomotor neurons converts the intestine in situ from an absorptive state to a secretory state with increased liquidity of the luminal contents.

Inhibitory inputs decrease the probability of secretomotor firing. The physiologic effect of inhibiting secretomotor activity is suppression of mucosal secretion. Postganglionic neurons of the sympathetic nervous system are one of the important sources of inhibitory input to the secretomotor neurons.[96,97] Submucosal somatostatinergic neurons are another source of inhibitory input.[96] Norepinephrine released from sympathetic axons acts at α_{2a}-noradrenergic receptors to inhibit the secretomotor neurons. Inhibition of secretomotor firing reduces the release of excitatory neurotransmitters in the crypts. The end result is conversion to an absorptive state with reduced secretion of water and electrolytes. Suppression of secretion in this manner is postulated to be part of the mechanism by which low-dose AT-II stimulates absorption in association with augmented intramural sympathetic nervous activity.

Results of electrophysiologic studies in secretomotor neurons suggest that AT-II enhances inhibitory sympathetic noradrenergic neurotransmission to secretomotor neurons and thereby suppresses mucosal secretion.[98] Exposure to AT-II depolarizes the membrane potential and elevates neuronal excitability in small numbers of myenteric neurons (~25%) and submucosal neurons (~32%). On the other hand, hyperpolarizing responses (ie, inhibitory responses) are evoked by AT-II in nearly one half of the neurons in both plexuses. The hyperpolarizing responses are suppressed by α_2-noradrenergic receptor antagonists, which suggests that the hyperpolarizing responses reflect stimulation of norepinephrine release from sympathetic neurons. Exposure to AT-II enhances the amplitude and prolongs the duration of noradrenergic IPSPs in secretomotor neurons and suppresses the amplitude of both fast and slow EPSPs. The selective AT-II_1 receptor antagonists ZD-7115 and losartan, but not a selective AT-II_2 receptor antagonist (PD-123319), suppress the actions of AT-II. Western blot analysis and reverse-transcription polymerase chain reaction find expression of AT-II_1 receptor protein and the messenger RNA transcript for the AT-II_1 receptor in the ENS. No expression of AT-II_2 receptor protein or messenger RNA can be found in the guinea pig ENS. AT-II_1 receptor IR is expressed by a majority of enteric neurons in the gastric antrum and small and large intestine.

The evidence suggests that formation of AT-II might have paracrine-like actions in the ENS, which would include alterations in neuronal excitability and facilitated release of norepinephrine from sympathetic postganglionic axons. The enhanced presence of norepinephrine is expected to suppress fast and slow excitatory neurotransmission in the enteric microcircuits and to suppress neurogenic mucosal secretion. Hard-dry stools and chronic constipation in some patients might reflect enhanced sympathetic nervous activity and elevated release of norepinephrine. In such cases, it might be predicted that treatment with an angiotensin-converting enzyme inhibitor would relieve the constipation.

Inflammation

Work to understand the actions of inflammatory/immune mediators in the ENS began with histamine in 1975, when exposure to it was found to excite neurons in the myenteric plexus of cat small intestine.[99] Since then, several putative mediators expected to be present in the inflamed bowel or to be released in response to sensitizing antigens in atopic bowel have been tested for their electrophysiologic actions on neuronal excitability and neurotransmission in the ENS. These include histamine,

5-HT, adenosine, interleukin (IL)-1β, IL-6, leukotrienes, prostaglandins, nitric oxide, and mast cell proteases, actions of which have been reviewed in detail.[38] Most act to mimic slow EPSPs in AH-type enteric neurons, an action that includes membrane depolarization, decreased membrane conductance, elevated excitability, and suppression of hyperpolarizing afterpotentials. Moreover, most of the inflammatory/immune mediators act to suppress fast nicotinic EPSPs and noradrenergic IPSPs. Elevated excitability occurs also in submucosal secretomotor neurons. The elevation of excitability, which occurs coincident with suppression of noradrenergic IPSPs and removal of sympathetic braking action on secretomotor neurons, undoubtedly underlies the neurogenic secretory diarrhea associated with inflammatory states and responses to allergens.

Animal models for intestinal inflammation, parasitic infection (eg, *Trichinella spiralis* or *Nippostrongylus brasiliensis*), and food allergy (eg, milk protein or ovalbumin) have proved useful for translating to the pathologic state of the whole bowel, the observations that have been obtained with experimental application of inflammatory/immune mediators to single enteric neurons.[38,100–106] Preparation of the inflammatory models involves rectal injection of agents (eg, trinitrobenzene sulfonic acid [TNBS], acetic acid, turpentine, or mustard oil), which results in local mucosal inflammation or transmural inflammation depending on the agent used.

Neuronal excitability of single enteric neurons is enhanced during the inflammatory phase of infection with *T spiralis* as compared with uninfected animals.[107,108] Decreased resting membrane potentials, increased membrane input resistance, decreased threshold for action potential discharge, and suppression of the amplitude and duration of hyperpolarizing afterpotentials occur in AH-type neurons in preparations from infected guinea pigs. The state of augmented excitability in AH-type neurons, which is found with electrophysiologic recording in single neurons, is reflected by increased cytochrome oxidase activity and expression of *c-Fos* IR.[108] Excitability in S-type enteric neurons is also elevated in preparations from *T spiralis*–infected animals, as reflected by elevated levels of spontaneous action potential discharge.[107]

Mast cells. Mastocytosis occurs in *T spiralis* infection, and the excitatory effects of application of *T spiralis* antigens to enteric neurons in preparations from infected guinea pigs in vitro reflect mast cell release of histamine and its excitatory action at histamine H_2 receptors on the neurons.[38,107] The situation is the same in the small and large intestine of guinea pigs that are sensitized to a food antigen. Exposure to the antigen evokes mast cell release of histamine, which acts at histamine H_2 receptors to elevate neuronal excitability. The overlay of histamine on the neural networks also acts at presynaptic inhibitory histamine H_3 receptors on postganglionic sympathetic nerve terminals to suppress release of norepinephrine.[109,110] Mast cells in the stomachs from the same animals do not become sensitized to the food antigen (ie, milk protein), and histamine has no excitatory action on neurons in the gastric myenteric plexus.[110]

Human mast cells. Mast cells from human intestine appear to behave in a manner similar to that of mast cells in the animal models. Mast cells, enzymatically dispersed from human intestine and maintained in culture, can be stimulated to degranulate and simultaneously release multiple mediators by cross-linking of their immunoglobulin E receptors with an antibody that binds the Fcϵ α chain.[111] The culture media, with the released mast cell products, are then centrifuged and stored frozen to await study of effects on enteric neurons. Application of supernatants containing mast cell secretory products to either guinea pig or human ENS preparations in vitro evokes excitatory responses in single neurons that are reminiscent of the effects of mast cell degranulation in food allergy and animal models for parasitic infection.[107–111] Unlike the findings in animal models, the products of human mast cell degranulation appeared not to suppress fast nicotinic neurotransmission in the ENS preparations, as determined by application of imaging technology with voltage-sensitive dyes.[111]

Chemically induced inflammatory models. A minor loss of enteric neurons occurs in the animal models with TNBS-induced colitis.[78] Elevated excitability of enteric neurons in this model is a constant finding when electrical and synaptic behavior are recorded with intracellular microelectrodes.[100,104] Hyperexcitability is especially prominent in AH-type neurons in the TNBS guinea pig model. AH-type neurons, which generally fire only once or not at all in response to long-lasting depolarizing current pulses in their resting state in the normal intestine, fire repetitively in response to the same depolarizing pulses in the TNBS-inflamed intestine.[104] Elevated excitability in the AH-type neurons in the TNBS models is associated with shortening of the action potential duration and suppression of the characteristic hyperpolarizing after-spike potentials. No statistically significant change in either the membrane potential or input resistance of the AH-type neurons has been reported for the TNBS model.[102,104]

Some of the electrophysiologic changes in AH-type neuronal behavior in the TNBS model are essentially the

same as the behavior during slow synaptic excitation and the actions of putative paracrine inflammatory mediators (eg, histamine, prostaglandins, platelet-activating factor, and so on).[38] A "cocktail" of inflammatory mediators is undoubtedly "flooding" the ENS microcircuitry in the TNBS model and can be postulated to account for the observed alterations in neuronal activity. Separate identification of each of the involved mediators remains as a project for the future. Nevertheless, Linden et al[103] reported that activation of cyclooxygenase and associated prostaglandin production was one of the factors associated with the hyperexcitability in the AH-type neurons. Prostaglandins are known to evoke slow EPSP-like responses in AH-type neurons when applied exogenously to freshly dissected preparations in vitro and mimic the electrophysiologic changes found in TNBS-induced colitis when stable analogues are applied over 2-day periods.[69,106,112]

Whereas fast nicotinic neurotransmission is found to be suppressed by mast cell degranulation during exposure to sensitizing antigens, fast and slow EPSPs are reported to be significantly larger in the TNBS-inflamed colon of guinea pigs.[102,104] Stimulus-evoked fast EPSPs in submucosal neurons lose some of their sensitivity to blockade by hexamethonium in S-type neurons in the inflamed intestine.[102] Fast EPSPs in the submucosal plexus of the inflamed intestine develop sensitivity to suppression by purinergic P2X and 5-HT_3 receptor antagonists that is not readily evident in normal controls. Presynaptic facilitation of neurotransmitter release has been proposed as the mechanism underlying augmentation of neurotransmission in the TNBS-inflamed mucosa.[102]

Postinfectious IBS. Following an acute bout of infectious enteritis, a significant proportion of patients develop IBS-like symptoms.[113–115] Hypochondriasis and adverse life events are reported to double the risk for development of postinfective IBS.[113–116] Nevertheless, the question of whether the association between acute infectious enteritis and IBS reflects low-level inflammation (eg, microscopic enteritis) and long-term exposure of the neural and glial elements of the ENS to elevated levels of 5-HT, histamine, or other inflammatory mediators is suggested but remains to be fully resolved.

Enteric Glial Cells

Enteric glial cells (EGCs) were, until recently, a generally overlooked component of the ENS. They are now becoming a focus of increasing attention in neurogastroenterology, especially in terms of mucosal protection, inflammatory responses, and signaling.[117]

EGCs express many of the properties of the astroglia in the CNS and represent one of the several criteria often evoked as justification for reference to the ENS as a "brain in the gut." Contrary to past assumptions, accumulating evidence suggests that EGCs are more than passive scaffolding that supports the neurons in ENS ganglia. When positioned outside of the ganglia in the lamina propria, they are associated with submucosal blood vessels and the mucosal epithelium. EGCs form a dense latticework of cells in close apposition to the basal side of the intestinal mucosal epithelium. Current evidence and concepts now interpret the EGCs as being actively involved in the integrated functions of the whole organ. They are believed necessary for the structural and functional integrity of the ENS and maintenance of the mucosal epithelial barrier and to be participants in inflammatory/immune responses.

Intestinal protective functions. Fulminant and fatal inflammation of the intestine, which is unrelated to bacterial overgrowth, occurs in transgenic mouse models after ablation of their EGCs.[118] The earliest pathologic sign in the mice is a submucosal vasculitis followed by inflammatory disruption of the mucosa. These observations were among the first to implicate EGCs in regulation of permeability of the vascular endothelium and the mucosal epithelium. Recent findings suggest that EGCs are important factors in the maintenance of the integrity of epithelial tight junctions and their role in establishing mucosal barrier function.[119,120] Perturbation of EGCs in animal models in vivo results in increases in paracellular permeability of the mucosal epithelium and changes in the expression of synthetic enzymes for neurotransmitters in enteric neurons (eg, nitric oxide synthase and choline acetyltransferase). Inclusion of EGCs in cocultures with intestinal epithelial cells (ie, Caco-2, HT29, and IEC-6 cells) increases electrical resistance as the epithelial cells become confluent in monolayers, which is indicative of sealing of "tight" epithelial cell junctions and tightening of the "epithelial barrier" to movement of larger molecules. EGCs also exert strong antiproliferative effects on the various epithelial cell lines in culture, and this is dependent on secretion of transforming growth factor β1. The presence of EGCs after 2 days in coculture with the Caco-2 epithelial cell line significantly increases the surface area of the monolayers in the absence of Caco-2 cellular hypertrophy or mitosis.

The influence of EGCs on the formation of an epithelial barrier appears to provide protection against transepithelial invasion by pathogens. In in vitro coculture models, the presence of EGCs decreases crossing of the epithelial barrier by *Shigella flexneri* and also suppresses the inflammatory response to this organism.[120]

Results obtained from studies in which EGCs are cocultured with enteric neurons suggest that the EGCs protect the neurons against neurodegeneration under inflammatory conditions (eg, elevation of nitric oxide levels). Cytokine signals involving EGCs are central events underlying the inflammation. Selective activation of EGCs by proinflammatory cytokines reflects their role in intestinal inflammation.[121,122] Exposure to proinflammatory cytokines activates *c-fos* expression in EGCs in vitro, and *c-fos* expression is known to be up-regulated in intestinal inflammation induced by intramural injection of formalin in rat colon.[123] Exposure of EGCs in purified primary cultures to IL-1β stimulates the synthesis and release of IL-6. This action of IL-1β is at IL-1 receptors that are expressed by the EGCs.[124] At the level of the ENS, IL-1β and IL-6 act synergistically both to excite submucosal secretomotor neurons and to suppress the braking action of norepinephrine release from sympathetic postganglionic terminals in submucosal ganglia.[124–126]

Signaling in glial networks. EGCs are linked one to another into networks by gap junctions that conduct ions and larger organic molecules.[127] Mechanical or chemical stimulation of a single glial cell in an EGC network in culture evokes a wave of Ca^{2+} release that travels from cell to cell throughout the network.[128] The propagation of the Ca^{2+} waves in the EGC networks appears as the same phenomenon in CNS astroglia in culture[129] and is reminiscent of the transmission of impulses in nerve fibers.

Like the networks of astroglia in the CNS, neurocrine, endocrine, or paracrine signaling to the EGC networks is suggested by the expression of receptors for a number of known signal molecules. EGC networks respond to ATP, uridine triphosphate, 5-HT, histamine, and bradykinin.[130] Like ENS neurons, EGC networks also express PAR-1 and PAR-2 and can respond to mast cell proteases with propagating Ca^{2+} waves.[74,75,131] Application of endothelin to EGC networks likewise stimulates endothelin B receptors to evoke elevation of intracellular Ca^{2+} levels. The dynamics of Ca^{2+} homeostasis in this case involve release from intracellular stores followed by capacitative Ca^{2+} entry into the cells from the extracellular milieu.[132]

GI Neuromotor Control

The smooth muscles of the GI tract are classified as unitary-type smooth muscle. Unitary-type smooth muscle contracts spontaneously in the absence of neural or endocrine influence and contracts in response to stretch. Electrical slow waves generated by interstitial cells of Cajal account for the spontaneous myogenic contractile behavior of the intestinal musculature.[133] The slow waves propagate from interstitial cells of Cajal into the intestinal circular muscle coat and trigger action potentials, which in turn initiate contractions. Abnormal interstitial cells of Cajal have been reported in achalasia of the lower esophageal sphincter, infantile hypertrophic pyloric stenosis, chronic intestinal pseudo-obstruction, Hirschsprung's disease, inflammatory bowel diseases, and slow-transit constipation.[134]

Gastric Motility

Functionally, the stomach consists of a proximal reservoir and distal antral pump with distinct differences in motility between the 2 regions. The muscles of the reservoir are adapted for maintaining continuous contractile tone (tonic contraction) and do not contract phasically, while the muscle of the antral pump contracts phasically. The spread of ring-like contractions in the antral pump propels the gastric contents toward the gastroduodenal junction. Dysrhythmias in the antral pump are associated with disturbed gastric emptying and nausea and vomiting that can occur in motion sickness, gastric ischemia, and pregnancy.[135]

The gastric reservoir has 2 primary functions. One is to accommodate the arrival of a meal, allowing the stomach to fill without a significant increase in intragastric pressure. The second is to generate a compressive force to drive the contents of the gastric reservoir into the propulsive motor activity of the antral pump.

The gastric musculature is innervated by both excitatory and inhibitory motor neurons of the ENS. The motor neurons are controlled by both efferent vagal nerves and intramural microcircuits of the ENS. Vagal efferent nerve fibers release acetylcholine at nicotinic postsynaptic receptors on both excitatory and inhibitory enteric motor neurons. Excitatory motor neurons release acetylcholine at postjunctional muscarinic receptors and substance P at neurokinin-1 receptors on the musculature. Relaxation is mediated by release of nitric oxide, ATP, and vasoactive intestinal peptide from the inhibitory motor innervation of the musculature. The relative balance of excitatory and inhibitory input to the musculature adjusts the volume and pressure of the reservoir to the amount of solid and/or liquid present while maintaining constant compressive forces on the contents.[136] During ingestion of a meal and during emptying of the meal, continuous adjustments in the volume and pressure within the reservoir are required. Integrative interactions between the brainstem and ENS in the form of vagovagal reflexes determine the minute-to-minute behavior of the reservoir.

Increased firing of excitatory motor neurons in the ENS, coordinated with decreased activity of inhibitory motor neurons, results in increased contractile tone in the reservoir, decreased volume, and increased intrareservoir pressure. Increased firing of inhibitory motor neurons, coincident with decreased activity of excitatory motor neurons, results in decreased contractile tone in the reservoir, expanded volume, and decreased intrareservoir pressure.[137] Inhibitory motor neurons in the gastric reservoir express the serotonergic 5-HT_1 receptor, which, when activated, stimulates neuronal firing and release of inhibitory neurotransmitters that relax the musculature of the reservoir. Stimulation of the 5-HT_1 receptors on inhibitory motor neurons by sumatriptan has reported efficacy in treatment of dyspeptic symptoms of early postprandial fullness and satiety.[138] Animal studies also suggest that the 5-HT receptor on gastric inhibitory motor neurons might be the 5-HT_1 receptor subtype.[139]

Neurally mediated decreases in tonic contracture of the musculature are responsible for relaxation of the gastric reservoir (ie, increased volume). Three kinds of relaxation are recognized. (1) Receptive relaxation is initiated by the act of swallowing. It is a reflex triggered by stimulation of mechanoreceptors in the pharynx followed by transmission over afferents to the dorsal vagal complex (ie, the nucleus tractus solitarius and dorsal motor nucleus of the vagus) and subsequent activation of efferent vagal fibers to inhibitory motor neurons in the gastric ENS. (2) Adaptive relaxation is triggered by distention of the gastric reservoir. It is a vagovagal reflex triggered by stretch receptors in the gastric wall, transmission over vagal afferents to the dorsal vagal complex, and efferent vagal fibers to inhibitory motor neurons in the gastric ENS. (3) Feedback relaxation is evoked by the presence of nutrients in the small intestine mediated by both vagal and enteric reflexes triggered by paracrine signaling actions of cholecystokinin and/or 5-HT on vagal afferent terminals.[140] Adaptive relaxation is impaired in patients who have experienced iatrogenic injury to the vagus nerves during procedures such as laparoscopic fundoplication surgery. The loss of adaptive relaxation following vagal injury is associated with a lowered threshold for sensations of fullness and pain during gastric filling. Descending influences from higher brain regions modulate vagovagal transmission and account for emotional and behavioral influences on gastric function (eg, during stress). These influences can extend throughout the GI tract.

Intestinal Motility

Four fundamental patterns of small intestinal motility are the interdigestive migrating motor complex (MMC) pattern, the postprandial pattern of mixing movements, power propulsion, and neurally programmed musculomotor quiescence (sometimes called physiologic ileus). The integrative microcircuitry of the ENS contains the programs for each of these patterns.

The MMC is a specific pattern of motor activity in the stomach and small intestine during fasting in most mammalian species, including humans.[141] The MMC continues in the small intestine after a vagotomy or sympathectomy but stops when it reaches a region of the intestine where the ENS has been interrupted.[142] Presumably, command signals to the enteric neural circuits in the form of neural synaptic input or overlay of a paracrine or hormonal signal substance initiate and sustain the MMC. Levels of the hormone motilin reach a peak in the plasma coincident with the onset of the MMC in the antrum.[143] The adaptive significance of the MMC appears also to include a mechanism for clearing indigestible debris from the intestinal lumen during the fasting state. Bacterial overgrowth in the small intestine is associated with absence of the MMC.[144,145] This condition suggests that the MMC may play a "housekeeper" role in preventing the overgrowth of microorganisms that might occur in the small intestine if the contents were allowed to stagnate in the lumen. Small intestinal bacterial overgrowth was recently proposed as the underlying pathophysiology of IBS symptoms.[146,147]

A mixing pattern of motility (segmentation) replaces the MMC when the small intestine is in the digestive state following ingestion of a meal. Volume and caloric content of the meal determine the duration of the postprandial motility pattern. Lipids, particularly medium-chain triglycerides, are most effective in suppressing the neural program for the MMC and calling up the program for the segmentation pattern of the digestive state.[148] During the postprandial pattern, peristaltic contractions, which propagate for only very short distances, account for the segmental appearance of the bowel on x-ray film. Signals transmitted from the brainstem by vagal efferent nerves to the ENS interrupt the MMC and initiate the neural mixing program during ingestion of a meal. Interruption of the MMC by a meal does not occur in extrinsically denervated segments of small intestine in dog models.[149] Blockade of vagal nerve conduction prevents the conversion from the interdigestive to the postprandial motility state in all regions of extrinsically innervated small intestine.[150,151]

The power propulsion motor pattern is relevant for understanding the symptoms of cramping abdominal pain, diarrhea and fecal urgency in infectious enteritis, inflammatory bowel disease, radiation-induced enteritis, and FGIDs. It is peristaltic propulsion characterized by strong, long-lasting contractions of the circular muscle

that propagate for extended distances along the small and large intestine. The circular muscle contractions during power propulsion are sometimes referred to as "giant migrating contractions" because they are considerably stronger than the phasic contractions during the MMC or mixing pattern.[152] Giant migrating contractions last 18–20 seconds and span several cycles of the electrical slow waves. They are a component of a highly efficient propulsive mechanism that rapidly strips the lumen clean as it travels rapidly over long lengths of bowel.

Power propulsion occurs in the retrograde direction during emesis in the small intestine and in the orthograde direction in response to noxious stimulation in both the small and large intestine. Abdominal cramping pain and sometimes diarrhea are associated with this motor pattern.[153] Application of irritants to the mucosa, the introduction of luminal parasites, enterotoxins from pathogenic bacteria, allergic reactions, and exposure to ionizing radiation each activate the powerful propulsive motor program. These characteristics suggest that power propulsion is a defensive adaptation for rapid clearance of noxious or threatening contents from the intestinal lumen. It also accomplishes mass movement of intraluminal contents in normal states, especially in the large intestine (eg, defecation).

Motility of the Anorectum and Pelvic Floor

The levator ani, puborectalis, and external anal sphincter muscles are of concern in consideration of defecatory mechanisms, fecal continence and incontinence, and pelvic pain. The levator ani are overlapping sheets of skeletal muscle that form the pelvic floor. The levator ani contract and relax in concert with the puborectalis and the external anal sphincter as components of a functioning unit necessary for the maintenance of fecal continence and normal defecation. The pelvic floor and anorectal musculature have properties like those of the tonic somatic muscles that maintain upright bodily posture against the forces of gravity.[154,155] Weakening of the musculature (eg, in advanced age) or traumatic damage to the musculature or its innervation (eg, during childbirth) can underlie fecal incontinence and disordered defecation.[156] Like other skeletal muscles, weakening of the pelvic floor musculature can often be corrected through targeted isometric exercise.[157]

The internal and external anal sphincters surround the anal canal. The external sphincter is skeletal muscle that contracts and closes the anal orifice. The internal anal sphincter is a modified extension of the circular muscle coat of the rectum. It is fatigue-resistant smooth muscle that, like other sphincteric muscles, contracts tonically to sustain closure of the anal canal. Timed activation of its inhibitory innervation relaxes muscle tension and transiently opens the sphincter to permit forward passage of luminal contents. Sphincteric achalasia occurs when ENS inhibitory motor neurons to the sphincter are lost or fail to function.

Tonic contraction of the internal anal sphincter and the puborectalis muscle blocks the passage of feces into the anal canal and maintains continence with small volumes in the rectum. Rapid influx of feces and consequent distention of the rectum activates the rectoanal reflex, which relaxes the internal sphincter and simultaneously contracts the external sphincter. The rectoanal reflex involves stimulation of distention receptors in the rectal wall and processing of the sensory information by neural networks in the ENS and spinal cord (see Visceral Pain and Sensation). Processing in the ENS leads to reflex excitation of inhibitory motor neurons to relax tension in the smooth muscle of the internal anal sphincter. Processing in the spinal cord leads to excitation of spinal motor neurons to contract the external anal sphincter. Conscious sensation of rectal fullness in the lower abdomen is experienced as the rectum fills and reflects CNS processing of input from mechanoreceptors in the pelvic floor musculature. Sensory physiology of afferents from the pelvic floor differs significantly from splanchnic afferents.[158,159] Pelvic mechanosensitive afferents respond to lower stimulus intensities, have larger response magnitudes, and adapt less completely to sustained stimulation. Pelvic floor afferents also differ from intestinal afferents by having a lack of sensitivity to application of inflammatory mediators. Most intestinal afferents are stimulated by application of bradykinin or ATP, while pelvic floor afferents are resistant to these agents and to stimulation by capsaicin.

Relaxation of the internal anal sphincter allows contact of the rectal contents with the sensory receptors in the lining of the anal canal. Sensory signals that reach the brain from the anal canal serve as an early warning of the possibility of a breakdown of maintenance of continence. Continence in this situation is maintained by reflex and conscious voluntary contraction of the external anal sphincter and puborectalis muscle. The external sphincter closes the anal canal and the puborectalis sharpens the anorectal angle to form a mechanical barrier to onward movement of the feces.

Power propulsion in diarrheal states (eg, diarrhea-predominant IBS or infectious enteritis) moves large volumes of liquid stool with high velocity into the rectum with potential for overwhelming the mechanisms of continence and the embarrassment of soiling.[153,160] Sensory detection of rectal distention by rapid influx of large volumes of liquid stool, transmission to the spinal

cord, and supraspinal processing of the information underlie the sensations of fecal urgency in diarrheal states.

Stress GI Function and Visceral Pain

Brain-gut interactions are reflected by the perturbations of GI motor and mucosal function and visceral sensitivity that are induced by various stressors.[161] In animals and humans, a number of stressors inhibit gastric and small intestinal transit while stimulating colonic motility.[79] Stress also elevates intestinal secretion of electrolytes, mucus, and H_2O coincident with enhanced permeability that permits penetration of antigens and commensal microbes into the lamina propria in rodents.[162,163] In a primate stress model, exposure to fecal antigens initiates inflammatory responses that resemble ulcerative colitis and a progression to colon cancer.[164] Genetic or environmental factors and previous visceral inflammation influence the outcome of acute stress on colonic barrier function, mucosal immunity, motor function, and visceral sensitivity.[165]

Psychological stress exacerbates the symptoms of IBS, of which cramping abdominal pain associated with urgency and explosive watery diarrhea predominate.[166] The association with stress is further reflected by observations that psychotherapy and treatment with low-dose antidepressants (eg, tricyclic antidepressants and selective serotonin reuptake inhibitors) are often effective.[167–169] The simultaneous impact of stress on colonic motor, secretory, and immune functions and the partial dependence on stimulation of CRF receptors in the brain and the ENS of animal models support a hypothesis that CRF neural signaling is a contributory factor in the stress-related exacerbation of symptoms in patients with IBS.

CRF and Stress

CRF and 3 related peptides (urocortin 1, 2, and 3) bind to CRF_1 and CRF_2, which are distinct G protein–coupled receptors.[170] CRF interaction with the pituitary CRF_1 receptor is essential for the glucocorticoid increase induced by stress.[171] Activation of brain CRF_1 receptors mediates stress-induced colonic motor response and visceral sensitization to colorectal distention.[79,161] Intestinal CRF_1 receptors on enteric cholinergic neurons also contribute to colonic motor responses to stress.[161] The augmentation of colonic mucosal secretion and disruption of mucosal barrier function induced by stress are also mediated by intramural CRF receptors and related degranulation of enteric mast cells.[161] IR for CRF is expressed in nerve cell bodies and fibers in both the myenteric and submucosal plexuses in most regions of the GI tract.[81] CRF-immunoreactive nerve fibers in the intestine are all derived from enteric neurons; neither sympathetic postganglionic fibers nor sensory afferent fibers express CRF IR. Only the CRF_1 receptor subtype is expressed by enteric neurons.[80]

Activation of central CRF-CRF_1 signaling has emerged as a key factor for understanding stress-induced behavioral changes in animal models.[172] These include anxiousness, impairment in cognitive performance and locomotor activity, altered sleep patterns, and addictive behaviors, all of which take place independently of the adrenal cortical endocrine response.[172] Likewise, in humans, stress-associated dysfunction of the CRF-CRF_1 neuronal circuitry in the brain is implicated in the onset and persistence of affective disorders such as anxiety, major depression, and early stress.[172] Comorbidity of IBS with anxiety and depression might be explained in the context of hyperactivity of CRF-CRF_1 signaling pathways in the brain. Activation of specific hypothalamic areas (eg, paraventricular nucleus) or pontine areas (eg, locus ceruleus and Barrington's nucleus) by exogenously applied CRF or by exposure to stress results in behavior symptomatic of anxiety and/or depression coincident with colonic motor dysfunction.[161] Administration of CRF_1 receptor antagonists alleviates these effects in rodents.[161] Of interest in this respect are reports that activation of neurons in the locus ceruleus by colorectal distention in rats involves stimulation of the CRF_1 receptor.[173] Increased firing of neurons in the locus ceruleus, in response to exteroceptive or interoceptive stressful input, results in widespread activation of noradrenergic neurons, which project to forebrain sites associated with arousal and focused attention.[174] Enhanced activity in these forebrain projections is postulated to underlie the stress-related alterations in perceptual threshold to colorectal distention and the hyperreactivity associated with stress in patients with IBS.[175] The collective evidence from animal models suggests that blockade of CRF_1 receptor signaling in the brain has the beneficial effect of preventing stress-related gut dysfunction and visceral hypersensitivity.[172]

Neuroimmunology

Several cell types of immune/inflammatory cells, which include polymorphonuclear leukocytes, lymphocytes, macrophages, dendrocytes, and mast cells, are present in continuously varying numbers in the intestinal mucosa, lamina propria, and smooth muscle. Each of these cell types may be situated in close association with the neuronal elements of the ENS, vagal nerve fibers, and spinal sensory nerves and

are the source of paracrine signals that initiate and modulate reflex responses.[75,176–178]

Enteric Mast Cells

Mast cells are equipped and strategically located to recognize foreign agents that threaten whole body integrity and to signal the ENS to program a defensive response.[38] Enteric mast cells express high-affinity receptors for immunoglobulin E antibodies or other immunoglobulins on their surfaces. Antigen recognition by antibodies bound to the sensitized mast cells triggers degranulation and release of the mast cell mediators. After release, the mediators become paracrine signals to the ENS, which responds by "running" a defense program designed to eliminate the antigen from the lumen. Copious secretion and increased blood flow followed by orthograde power propulsion of the luminal contents are the behavioral components of the program.

Enteric mast cells are used also by the CNS as a link for sending chemical signals to the ENS.[1,2,38,41] This is a brain-gut interaction in which central psychological status might be linked to irritable states of the digestive tract by way of mast cell degranulation and release of mediators. Mast cell degranulation evoked by psychological stress activates the ENS "alarm program" to produce the same symptoms of diarrhea and abdominal distress as antigen-evoked degranulation.[38] Colonic mucosal biopsy specimens from patients with IBS contain elevated numbers of mast cells.[179–182] In experimental animals, degranulation of enteric mast cells results in a reduced threshold for pain responses to intestinal distention, and this is prevented by treatment with mast cell–stabilizing drugs.[183,184]

Mast Cell Mediators

As mentioned in an earlier section, several of the mast cell–derived mediators described have neuropharmacologic actions on the electrical and synaptic behavior of neurons in the ENS. Histamine is one of the key mediators in orchestrating intestinal defensive programs and serves as an example of the way mast cell mediators influence ENS function and afferent signaling. One action of histamine in the ENS occurs at the level of neuronal cell bodies, where it gives rise to long-lasting excitation mediated by histamine H_2 receptors in guinea pigs.[185] Another is at fast excitatory nicotinic synapses, where it acts at presynaptic inhibitory histamine H_3 receptors to suppress cholinergic synaptic transmission.[186] A third action is to prevent inhibition of secretomotor-evoked mucosal secretion by the sympathetic innervation.[187] Histamine also acts on extrinsic splanchnic afferents to evoke an H_1 receptor–mediated excitation leading to augmented sensory input to the CNS.[188] Mast cells in colonic mucosal biopsy specimens from patients with diarrhea-predominant IBS release more histamine than normal subjects.[181] Elevated release of histamine onto the neural networks that control the secretomotor innervation of the intestinal crypts in this subset of patients with IBS might lead to secretory diarrhea like that associated with infectious agents and food allergies. Histaminergic receptor antagonists have been used effectively to treat watery diarrhea associated with mastocytosis and microscopic colitis.[189] The H_2 receptor antagonist cimetidine was used effectively for treatment of diarrhea associated with short-bowel syndrome in patients with Crohn's disease.[190]

Serotonin and FGIDs

Serotonin is the predominant paracrine mediator expressed by mucosal enterochromaffin cells.[191] Many of the GI responses to 5-HT arise from activation of receptors on enteric neurons and sensory afferent neurons. Several distinct families of receptors mediate the excitatory actions of 5-HT. Metabotropic G protein–coupled receptors of the 5-HT_4 and 5-HT_{1P} subtype and ionotropic 5-HT_3 receptors mediate the actions of 5-HT on enteric neurons and sensory afferents. A selective antagonist for the 5-HT_7 receptor subtype suppresses stimulus-evoked slow EPSPs in the ENS and the slow EPSP-like action of exogenously applied 5-HT.[192] Slow EPSP-like actions of 5-HT were attributed to stimulation of 5-HT_{1P} receptors in the past.[62,193–196] The 5-HT_{1P} receptor now appears to be a hetero-oligomeric combination of 5-HT_{1B} and dopamine D_2 receptors.[197] Elevated levels of 5-HT in the hepatic portal circulation in the postprandial state reflect stimulated release from mucosal enterochromaffin cells. The postprandial release of 5-HT is reported to be augmented in patients with IBS and has been suggested to underlie the IBS symptoms of cramping abdominal pain, diarrhea, and fecal urgency in patients with diarrhea-predominant IBS.[116] This observation is reinforced by findings of elevated numbers of enterochromaffin cells in rectal mucosal biopsy specimens from patients with IBS.[180]

Application of 5-HT evokes increased firing in extrinsic afferents via 5-HT_3 receptors that can be blocked by selective antagonists (eg, alosetron).[3,5,198] Intramural terminals of both spinal and vagal afferents express 5-HT_3 receptors. Reported efficacy of alosetron in the treatment of abdominal pain and discomfort in the diarrhea-predominant form of IBS in women suggest that these symptoms reflect disordered endogenous release of 5-HT.[57] Similarly, ligands acting at 5-HT_4 receptors might also modulate afferent sensitivity[199] and may con-

tribute to the efficacy of such ligands in constipation-predominant IBS.[200]

Serotonergic signaling in the mucosa and the ENS is terminated by a transmembrane 5-HT reuptake transporter called SERT.[201] The mucosal epithelium and enteric neurons express SERT. Mucosal SERT expression is reported to be decreased in experimental inflammation, constipation-predominant IBS, diarrhea-predominant IBS, and ulcerative colitis.[200] Potentiation of the action of 5-HT due to decreased expression of SERT and persistence of 5-HT at its receptors might account for the discomfort and diarrhea in diarrhea-predominant IBS. Symptoms, which are reminiscent of the alternation of diarrhea and constipation in a subgroup of patients with IBS, are found in transgenic mice that lack SERT.[200]

Genetic polymorphisms in the DNA promoter region for SERT are associated with psychogenic disorders (eg, depression, schizophrenia, and bipolar disorder) and may underlie expression of one or the other of the IBS phenotypes (constipation, diarrhea, or alternator).[201–205] Genetic polymorphisms at the SERT promoter appear to influence the response of patients with diarrhea-predominant IBS to treatment with a 5-HT_3 receptor antagonist.[206]

Future Directions

Basic translational research in the immediate and extended future can be expected to maintain ongoing progress in each of the following areas:

1. Integration of CNS imaging technology and classic neurophysiologic and neuropharmacologic approaches for improved understanding of the neurobiology of the brain-gut axis.
2. Continued mechanistic focus on the basic science of visceral hypersensitivity and pain that includes the molecular basis for peripheral sensitization of sensory receptors by inflammatory mediators, selectivity of central pain-related transmission pathways, and higher-order central processing of nociceptive information from the viscera.
3. Expanded investigation of the neuroendocrine pathways, which connect the brain with the gut and are responsible for alteration of function during psychogenic stress.
4. Application of genomic chip technology in searches for genetic polymorphisms in receptors, enzymes, and steps in signal transduction cascades in elements of the ENS.
5. Focus on identification of drug targets on neural elements of the ENS and CNS and on nonneural cell types, such as mast cells and enterochromaffin cells, which release substances that alter the activity of neurons.[21,25,26,32,73,86,175,184]

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