EJ106199900201

Review Article

Neurotransmission at the Interface of Sympathetic and Enteric Divisions of the Autonomic Nervous System

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Abstract

The sympathetic and enteric divisions of the autonomic nervous system are interactive in the determination of the functional state of the digestive tract. Activation of the sympathetic input suppresses digestive function primarily through release of norepinephrine at its synaptic interface with the enteric nervous system. The enteric nervous system functions like an independent minibrain in the initiation of the various programmed patterns of digestive tract behavior and moment-to-moment control as the neural microcircuits carry-out the behavioral patterns. Most of the postganglionic projections from sympathetic prevertebral ganglia terminate as synapses in myenteric and submucous ganglia of the enteric nervous system. Two primary actions of the sympathetic input are responsible for suppression of motility and secretion. First is presynaptic inhibitory action of norepinephrine to suppress release of neurotransmitters at fast and slow excitatory synapses in the enteric neural microcircuits and this effectively shuts-down the circuit. Second is inhibitory synaptic input to submucosal secretomotor neurons to the intestinal crypts. The alpha$_2$ adrenergic receptor subtype mediates both actions. Axons of secretomotor neurons to the crypts bifurcate to innervate and dilate the submucosal vasculature. Dilitation of the vasculature increases blood flow in support of increased secretion. Sympathetic inhibitory input to the secretomotor neurons therefore suppresses both secretion and blood flow. Activation of the sympathetic nervous system cannot explain the symptoms of secretory diarrhea and abdominal discomfort associated with psychologic and other forms of stress. Current evidence suggests that brain to mast cell connections account for stress-induced gastrointestinal symptoms. Degranulation of enteric mast cells by neural inputs releases inflammatory mediators that enhance excitability of intestinal secretomotor neurons while suppressing the release of norepinephrine from postganglionic sympathetic axons. This is postulated to underlie the secretory diarrhea and abdominal discomfort associated with stress.

Key Words: intestine, gastrointestinal, enteric nervous system, myenteric plexus, submucous plexus, synaptic transmission, norepinephrine, stress, immune system

Introduction

The autonomic nervous system consists of sympathetic, parasympathetic and enteric divisions. Cell bodies of efferent parasympathetic neurons are located in the dorsal vagal complex of the medulla oblongata and the sacral region of the spinal cord. The enteric nervous system is an independent integrative system within the walls of the digestive tract and is often called the "brain-in-the gut" in recognition of its unique central nervous-like functions (28, 63). The sympathetic division of the autonomic nervous system, which together with the enteric nervous system is the central focus of this review, is positioned in the thoracic and lumbar regions of the spinal cord. Efferent fibers leave the intermediolateral cell column of the spinal cord in the ventral roots to make their first synaptic connections with neurons in prevertebral sympathetic ganglia located in the abdomen. The prevertebral ganglia are the coeliac,

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Received July 7, 1999; Revised August 12, 1999; Accepted August 12, 1999.

the superior mesenteric and the inferior mesenteric ganglia. Cell bodies in the prevertebral ganglia project axons to the digestive tract where they form synapses with neurons of the enteric nervous system and innervate the blood vessels, mucosa and specialized regions of the musculature.

Sympathetic nervous transmission to the gastrointestinal tract increases during physical exertion and environmental and/or psychogenic stress. Increased sympathetic activity acts to shunt blood from the splanchnic to the systemic circulation. This is significant because as much as 25 percent of the cardiac output can be perfusing the splanchnic circulation. Shunting of blood from the splanchnic circulation occurs coincident with suppression of digestive functions including motility and secretion. Release of norepinephrine from sympathetic postganglionic neurons is the principal mediator of these effects. Norepinephrine acts directly on sphincteric muscles to increase tension and keep the sphincter closed during shut-down of motility. Evidence discussed in this review suggests that action of norepinephrine on neural elements of the enteric nervous system is primarily responsible for sympathetic inactivation of motility and mucosal secretion.

Apart from relaying sympathetic information from the spinal cord to the digestive tract, sympathetic prevertebral ganglia are components of pathways for rapid transfer of signals between separated regions of bowel (Fig. 1). Signal transfer within the enteric nervous system seldom travels more than a few centimeters before encountering a synapse. Consequently, signaling over the long distances found in the small and large intestine would be slow and susceptible to interruption. Connections that relay information through prevertebral ganglia are adaptations that overcome this "bottleneck". Exchange of information between widely separated regions of bowel occurs over extraintestinal pathways that bypass the synaptic circuits within the intramural nervous system. The extraintestinal pathways consist of projections from intramural neurons that form synapses with postganglionic sympathetic neurons in prevertebral ganglia. The postganglionic neurons in turn transmit the information to the bowel. Projections to the prevertebral ganglia can be derived from both spinal sensory neurons with cell bodies in dorsal root spinal ganglia and sensory receptors in the gut wall or from neurons with cell bodies in the myenteric plexus of the enteric nervous system (45, 50).

A function of the intestinal bypass routes through prevertebral ganglia was illustrated effectively by experiments of Szurszewski and Krier (51). The experimental set-up consisted of two segments of isolated guinea-pig colon connected only by the

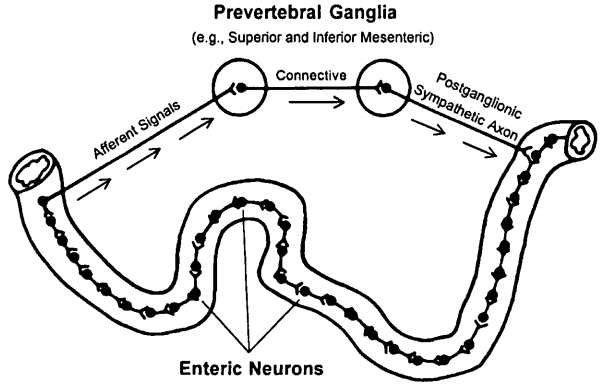

Fig. 1. Prevertebral sympathetic ganglia have neural connections for rapid transfer of signals between separated regions of the intestine. Afferent signals to the ganglia from one region of intestine are relayed as inhibitory signals in postganglionic sympathetic fibers to another region. The prevertebral ganglia and their connections provide extraintestinal pathways that bypass the synaptic circuits of the enteric nervous system and thereby increase the speed of communication between widely separated regions of bowel.

intermesenteric nerve and between the superior and inferior mesenteric ganglia. When the orad segment was distended, spontaneous contractile activity in the caudad section was stopped and then resumed when the distension was removed. Sectioning of the intermesenteric nerve during a second period of distension of the orad segment abolished the inhibition. This was called the "colo-colonic inhibitory reflex" and now generally recognized throughout the intestinal tract as "intestino-intestinal inhibitory reflexes".

Localization of Norepinephrine

Histochemical techniques for localization of monoamines reveal that sympathetic postganglionic axons in the myenteric plexus have bulbous expansions appearing every 1-3 μm along the fiber and that the varicosities contain high concentrations of catecholamines (15,30,36). Autoradiographic observations on the uptake of tritium-labeled norepinephrine indicate that most of the uptake is in enteric ganglia (33). Norepinephrine content in the myenteric plexus of guinea-pig small intestine ranges from 0.5 to 0.9 μg/g wet wt. (19). No epinephrine is detected in the enteric nervous system. In mammals, including humans, noradrenergic varicose axons appear in the myenteric plexus and removal of prevertebral ganglia results in complete disappearance of catecholamine flourescence (16, 23; Fig. 2). Most indications are that catecholamines are not synthesized by enteric neurons. Nevertheless, catecholamine-containing neurons have been reported for guinea-pig proximal colon (10) and cat small bowel (27).

The early results of Costa and Furness (9) did not show discrete synaptic junctions between

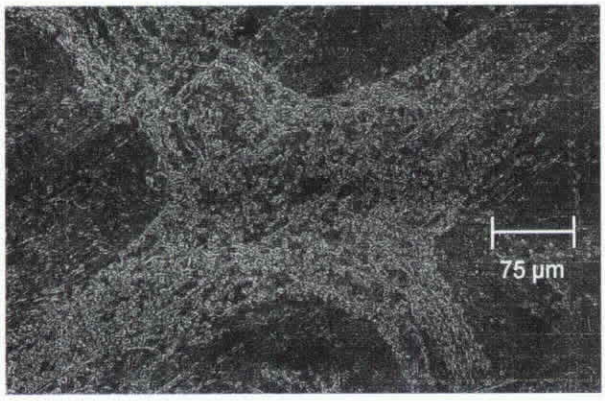

Fig. 2. Sympathetic postganglionic neurons provide extensive innervation in ganglia of the enteric nervous system. Immunohistochemical localization of tyrosine hydroxylase is shown to be restricted mainly to ganglia of the myenteric plexus in a whole-mount preparation from human ileum.

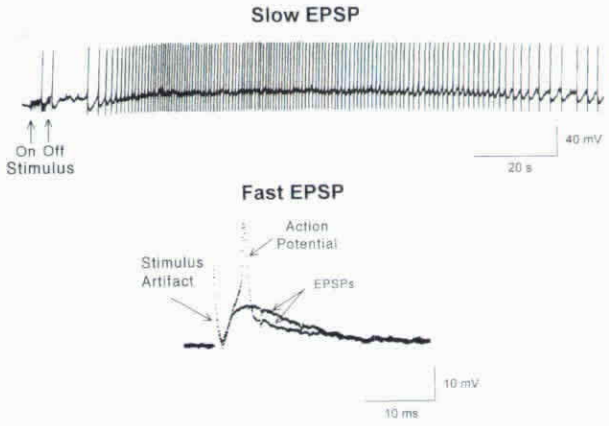

Fig. 3. Examples of slow and fast excitatory postsynaptic potentials (EPSPs) in enteric neurons. Slow EPSPs are slowly activating depolarizing potentials with enhanced excitability evidenced by train-like spike discharge lasting for several seconds to minutes. Fast EPSPs are depolarizing potentials occurring within milliseconds. Two superimposed fast EPSPs are shown; one reached threshold for triggering an action potential, the other did not. The probability of fast EPSPs reaching spike threshold is greatly increased during a slow EPSP.

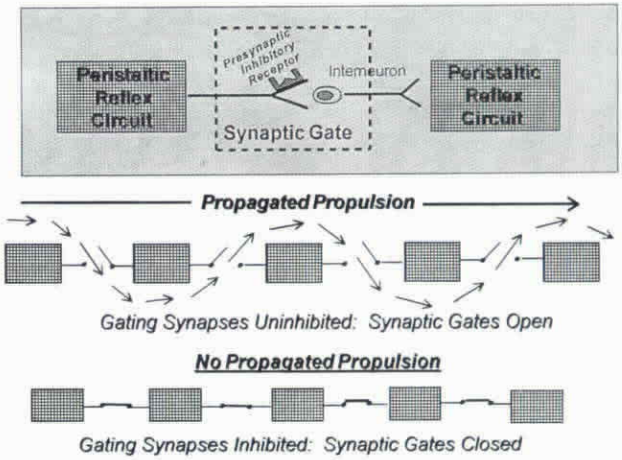

Fig. 4. Presynaptic inhibition accounts for interneuronal gating of the spread of neural signals in the enteric nervous system. Activation of presynaptic receptors (e.g., by norepinephring) suppresses release of neurotransmitters at enteric synapses. Suppression of transmitter release prevents transmission and thereby "closes the gate" for onward transfer of information. The distance over which peristaltic propulsion propagates is determined by the number of blocks of the basic peristaltic circuit that is signaled to action in sequence which in turn is determined by conduction at synaptic transfer points between blocks. If all connecting synapses between blocks of circuitry are inhibited, the "gates are closed" and no propagated propulsion occurs. If all gating synapses are uninhibited in a length of bowel, the "gates are open" and propagated propulsion occurs over the entire length of the segment.

catecholaminergic varicosities and enteric ganglion cells; instead, they showed *en passant* relationships which suggested that the norepinephrine diffuses within the intraganglionic space to reach its target and that the specificity of noradrenergic action on enteric nerve elements is not determined by their physical relationships to each other, but by the type of receptor on the neural elements. On the other hand, Manber and Gershon (31) reported ultrastructural evidence for reciprocal adrenergic-cholinergic axo-axonal synapses in enteric ganglia.

Noradrenergic Presynaptic Inhibition

Presynaptic inhibition is the term for mechanisms that suppress release of neurotransmitters from axons. It was described first for the enteric nervous system by Hirst and McKirdy in 1974 (21). Presynaptic inhibition is produced by the action of chemical messengers acting at receptors located on axons at release sites for neurotransmitters. It is a significant synaptic event within the enteric microcircuits of the stomach, small and large intestine and rectum of the guinea-pig (44, 52, 53, 59) where it occurs at both fast and slow excitatory synapses in the circuits (Fig. 3).

Presynaptic inhibition in the enteric nervous system operates normally in two important ways. First, it is a mechanism for selectively shutting-down and deenergizing a microcircuit. A mayor component of shut-down of gut function by the sympathetic nervous system involves the presynaptic inhibitory action of norepinephrine; this occurs simultaneously with the inhibition of secretomotor function discussed below.

Presynaptic inhibition underlies gating functions in the microcircuits and this is another aspect of physiologic importance. Gating refers to control of distance of spread of a behavioral event (e.g., peristaltic propulsion) along a length of intestine (Fig. 4). Events, such as mixing movements occur

over short lengths of intestine, whereas other motor events such as intestinal power propulsion travel over long segments of bowel when triggered by sensitizing antigens or noxious mucosal stimulation. Axons of enteric interneurons project only for short distances along the longitudinal direction of the intestine before ending as a synapse with another neuron. The longest projections are at most a few centimeters. This suggests that the spread of neuronal signals for propagated motor events must cross many synapses for propagation of the event to progress along the intestinal length. Moreover, each consecutive synapse must transmit unfailingly for propagation to continue. Presynaptic inhibition is an effective mechanism for stopping transmission at synapses. Consequently, activation of presynaptic receptors in the local neural networks within a given region of bowel brings a propagating event to a halt. Virtually all of the synapses in the neural circuitry of the small and large intestine and gastric antrum have presynaptic inhibitory receptors at the release sites for the neurotransmitters. This presents a continuous matrix of sites along the bowel where presynaptic inhibition can act to gate the distance, as well as the direction of travel of neural signals within the neural plexuses.

Norepinephrine acts at presynaptic $alpha_{2a}$ receptors to suppress transmission at both slow and fast excitatory synapses and at excitatory neuromuscular junctions in the gut (46, 49). Electrical stimulation of sympathetic postganglionic axons in the periarterial mesenteric nerves suppresses nicotinic fast excitatory postsynaptic potentials (fast EPSPs) in myenteric ganglion cells without altering responses to exogenously applied acetylcholine (21, 35). Application of norepinephrine or other $alpha_2$ agonists (e.g., clonidine) in *in vitro* studies results in suppression of fast EPSPs, but does not affect nicotinic depolarizing responses to micropressure pulses of acetylcholine (Fig. 5). These observations rule-out a postsynaptic site of action and fulfill criteria for presynaptic inhibition of acetylcholine release at the fast nicotinic synapse. Likewise, norepinephrine fulfills the criteria for a presynaptic inhibitory action by suppressing stimulus-evoked slow EPSPs without any effects on the postsynaptic actions of substance P, serotonin or muscarinic action of acetylcholine all of which mimic slow synaptic excitation when applied to enteric neurons. As expected for a presynaptic action, norepinephrine does not abort a slow EPSP in progress, but blocks ability to evoke subsequent EPSPs by electrical stimulation of interganglionic connectives in the myenteric plexus (64).

Norepinephrine reduces release of serotonin and substance P from isolated intestinal segments, consistent with presynaptic suppression of slow synaptic excitation (3, 25). Suppressive effects of

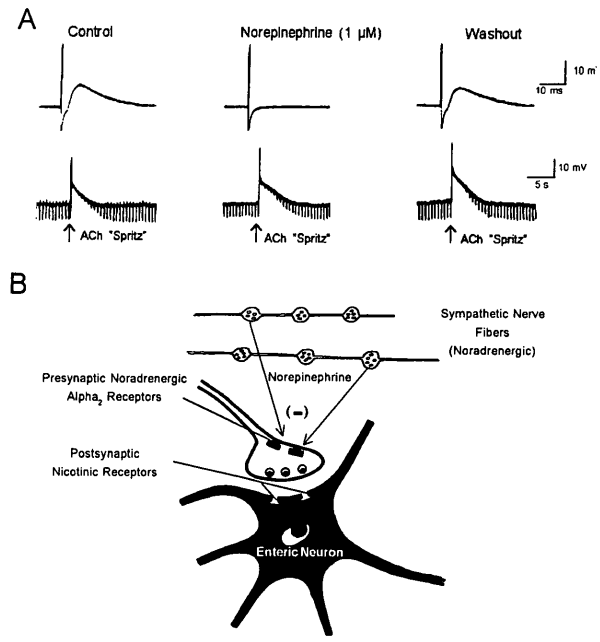

Fig. 5. Norepinephrine acts presynaptically to suppress release of acetylcholine (ACh) at nicotinic synapses in the enteric nervous system. (A) Effect of norepinephrine on the fast EPSP and the postsynaptic response to ACh applied by micropressure ejection from a fine-tipped pipette (i.e., "spritz). Upper traces are fast EPSPs evoked by stimulation of and interganglionic connective in the intestinal myenteric plexus and the lower traces are responses to ACh. Application of norepinephrine in the bathing solution suppressed the fast EPSP without affecting the response to ACh. This fulfills criteria for presynaptic inhibitory action of norepinephrine. Downward deflections on the lower traces are electrotonic potentials produced by intraneuronal injection of constant current hyperpolarizing pulses. Decreased amplitude of the electrotonic potentials during ACh depolarization reflect a decrease in the neuronal input resistance. (B) Diagramatic representation of relations of sympathetic postganglionic nerve fibers to synaptic transmission in the enteric nervous system. *En passant* release of norepinephrine from sympathetic axons acts at presynaptic receptors to suppress the release of neurotransmitters at enteric synapses.

norepinephrine on acetylcholine release from intestinal segments *in vitro* are well documented as evidence of presynaptic or prejunctional inhibitory action (13, 26, 38, 56). By suppressing the amount of acetylcholine and noncholinergic excitatory neurotransmitters released at synapses in the enteric neural networks and at neuromuscular junctions, norepinephrine can suppress motility by reducing the amount of excitatory neurotransmitter that reaches the musculature.

Noradrenergic Inhibition of Secretomotor Neurons

Secretomotor neurons are excitatory motor neurons in the submucous plexus that innervate the crypts of Lieberkuhn in the small and large intestine (Fig. 6). They are uniaxonal neurons with

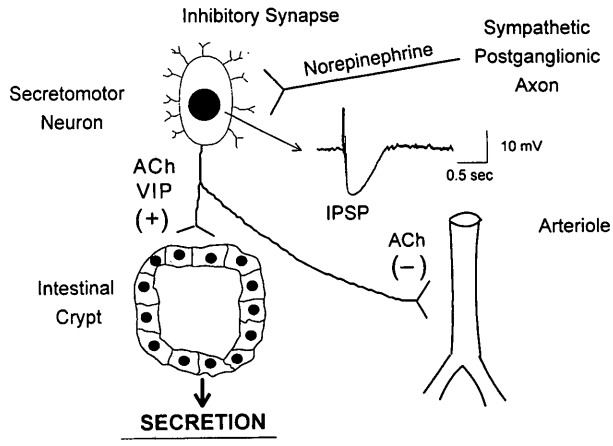

Fig. 6. Intestinal crypts of Lieberkuhn are innervated by secretomotor neurons. Secretomotor neurons release vasoactive intestinal peptide (VIP) and/or acetylcholine (ACh) to stimulate secretion from the crypt. Axon collaterals from secretomotor neurons innervate submucosal blood vessels. Release of ACh from the collaterals dilate submucosal arterioles and increase mucosal blood flow in support of stimulated secretion. Secretomotor neurons receive inhibitory synaptic inputs from sympathetic postganglionic neurons and from other neurons in the enteric microcircuits. Activation of the inhibitory synapses evoke inhibitory postsynaptic potentials (IPSP) in the secretomotor neurons. (Modified from: J.D. Wood, "Neurogastroenterology: Perspectives on a Supspeciality". In *Gastrointestinal Function*, Regulation and Disturbances, Excerpta Medica, Ltd., Tokyo, 1966, p. 11.)

characteristic Dogiel Type I morphology (4). When activated, they evoke secretion of water, electrolyte and mucus into the intestinal lumen. Most evidence suggests that acetylcholine and vasoactive intestinal polypeptide are the primary neurotransmitters released by these neurons (7, 8). The receptors for acetylcholine at the neuroepithelial junctions are muscarinic.

Dilation of submucous arterioles increases flow in support of elevated mucosal secretion and is mediated by intrinsic vasomotor neurons in the small intestinal submucous plexus of the guinea-pig (5, 34). Acetylcholine acting at the M_3 muscarinic receptor subtype is the primary neurotransmitter responsible for vasodilation (5, 34). The cholinergic receptors are located on the endothelium with endothelial release of nitric oxide being the vasodilatory mediator at the vascular muscle (1).

A significant component of vasodilatation of submucosal arterioles in the colon is noncholinergic. Vasoactive intestinal peptide, substance P and calcitonin gene-related peptide are the candidate neurotransmitters for these noncholinergic vasodilatory responses (1, 5, 24, 34). Part of the substance P innervation is derived from neurons with cell somas in the myenteric plexus and projections that pass through the circular muscle to the submucosa (47).

Submucosal vasomotor neurons and secretomotor neurons appear to be one and the same. Bifurcation of the axons of these neurons project both to the mucosa and arterioles (Fig. 6; A. Surprenant, *personal communication*). This achieves physiological conservation through utilization of the same population of motor neurons to ensure coordination of responses of two effector systems. Coupling of increased blood flow in support of stimulated secretion is achieved efficiently and parsimoniously by a set of common motor neurons that release neurotransmitters to simultaneously simulate secretion and increase blood flow. This organization predicts that circumstances in which secretomotor/vasomotor neurons are abnormally excited (e.g., VIPomas, food allergies and intestinal inflammation) will lead to secretory diarrhea; whereas, suppression of firing of the secretomotor neurons (e.g., sympathetic input described below and opioid antidiarrheal drugs) will lead to a constipational state.

Secretomotor neurons receive synaptic input from sympathetic postganglionic noradrenergic axons. Stimulation of the sympathetic fibers evokes slow inhibitory postsynaptic potentials (slow IPSPs) in the secretomotor neurons (Fig. 6). The slow IPSPs decrease the probability of spike discharge by the secretomotor neurons with the physiologic effect of suppression of mucosal secretion and associated submucosal blood flow. Norepinephrine released from the sympathetic axons acts at alpha$_2$ noradrenergic receptors to hyperpolarize and suppress the excitability of the secretomotor neurons (37). The end result is suppression of secretion of electrolytes, H_2O and mucus from the crypts. This is part of the mechanism involved in sympathetic nervous shutdown of gut function in homeostatic states during which blood is shunted from the mesenteric to the systemic circulation.

Immuno-Sympathetic Interactions

The intestinal tract is colonized by populations of immune/inflammatory cells that are constantly changing in response to luminal conditions and during pathophysiological states. In the colon, the mucosal immune system is exposed to one of the most contaminated of bodily interfaces with the outside world. The system is continuously exposed to dietary antigens, microorganisms and toxins. Physical and chemical barriers at the epithelial interface do not exclude the large antigenic load in its entirety, causing the mucosal immune system to be chronically challenged.

Studies in antigen sensitized animals suggest that the enteric immune system communicates directly with both the enteric (ENS) and sympathetic nervous

systems in ways that may be normal or pathological (6, 39, 48, 55, 58, 50, 62). The communication results in adaptive behavior of the intestine in response to conditions in the lumen that are threatening to the functional integrity of the whole animal. Communication is chemical (paracrine) and involves specialized sensing functions of the immune cells for specific antigens together with the capacity of the intestinal minibrain (i.e., ENS) for meaningful interpretation of the signals. Flow of information in immuno-neural integrative function starts with immune detection and signal transfer to the ENS. The enteric minibrain interprets the signal and responds by calling up from its library of stored programs, a specific program of coordinated mucosal secretion, blood flow and propulsive motility that functions to clear the antigenic threat from the intestinal lumen. Side effects of the program are symptoms of abdominal pain and diarrhea.

The enteric immune system becomes sensitized by foreign antigens in the form of food products, toxins and invading organisms. Once the system is sensitized, a second exposure to the same antigen triggers predictable integrated behavior of the intestinal effector systems (17, 55, 58, 60). Neurally coordinated activity of the musculature, secretory epithelium and blood vasculature results in organized behavior of the whole bowel that rapidly expels the antigenic threat. Recognition of an antigen by the sensitized immunoneural system leads to activation of a specialized propulsive motor program that is integrated with copious secretion of water, electrolytes and mucus into the intestinal lumen (48, 54, 55, 58, 60). Detection by the enteric immune system and signal transmission to the enteric minibrain initiates the defensive behavior which is analogous to emetic defense in the upper digestive tract. The neurally organized pattern of muscle behavior in response to an offensive antigen in the sensitized intestine is called power propulsion (63). This specialized form of propulsive motility forcefully and rapidly propels any material in the lumen over long distances and effectively empties the lumen. Its occurrence is associated with cramping lower abdominal pain and diarrhea (11, 12, 40).

Power propulsion is one of the neural programs contained in the library of programs stored in the enteric nervous system. Output of the program reproduces the same stereotyped motor behavior in response to radiation exposure, mucosal contact with noxious stimulants and antigenic detection by the sensitized enteric immune system (43). The neural program for power propulsion incorporates connections between myenteric and submucous plexuses that coordinate mucosal secretion with the motor behavior. The program is organized to stimulate

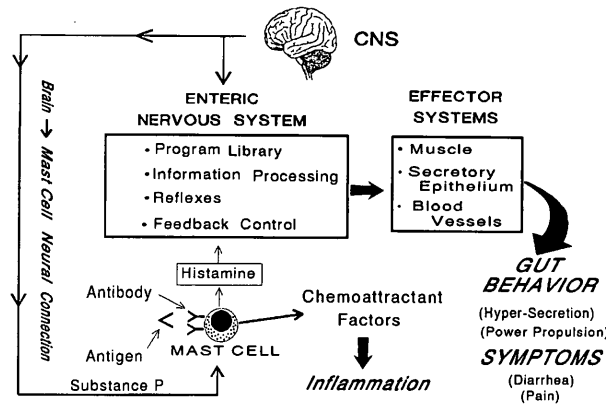

Fig. 7. Conceptual model for enteric neuro-immuno physiology. The ENS is a minibrain located in close apposition to the gastrointestinal effectors it controls. Enteric mast cells are in position to detect foreign antigens and signal their presence to the ENS. Stimulated mast cells release several paracrine mediators simultaneously. Some of the mediators signal the ENS while others act as attractant factors for polymorphonuclear leukocytes responsible for acute inflammatory responses. The ENS responds to the mast cell signal by initiating a program of coordinated secretion and propulsive motility that expels the source of antigenic stimulation from the bowel. Symptoms of abdominal pain and diarrhea result from operation of the neural program. Neural inputs to mast cells from the brain stimulates simultaneous release of chemoattractant factors for inflammatory cells and chemical signals to the ENS with effects that mimic antigenic stimulation. (Modified from: J.D. Wood, "The Enteric Neuroimmune System". In *News in Physiological Sciences*, 12: 245, 1997.)

copious secretion that flushes the mucosa and suspends the offensive material in solution in the receiving segment ahead of the powerful propulsive contractions, which empty the lumen. The overall adaptive significance is rapid excretion of material recognized by the immune system as threatening.

Several kinds of immune/inflammatory cells including lymphocytes, macrophages, polymorphonuclear leukocytes and mast cells are putative sources of paracrine signals to both the enteric nervous system and the projections to the enteric nervous system of postganglionic sympathetic fibers. Signaling between mast cells and the neural elements of the local microcircuits is the best understood. Intestinal mast cells proliferate during exposure to threatening invaders (e.g., the parasitic nematode *Trichinella spiralis*). Following an initial exposure to the invader, immunoglobulins specific for the parasite are bound to receptors on the mast cells where they confer memory for recognition of the sensitizing antigens. Binding and cross linking of the antigens with the antibodies triggers degranulation of the mast cells. Degranulation releases a variety of paracrine messengers which may include serotonin, histamine, prostaglandins, leukotrienes, platelet-

activating factor and cytokines (Fig. 7).

Two actions of histamine (61), prostaglandins (22), interleukin 1β and interleukin 6 (65), platelet-activating factor (66) and tumor necrosis factor alpha (67) are identified as factors responsible for the secretory diarrhea and motor behavior associated with various forms of enteritis. One of the actions is direct excitation of secretomotor neurons to the intestinal crypts and/or interneurons that provide excitatory synaptic drive to the secretomotor neurons (Fig. 6). Second is the presence of presynaptic inhibitory receptors for the inflammation-related mediators at release sites for norepinephrine on postganglionic sympathetic neurons. This inhibitory action on noradrenergic transmission to the secretomotor neurons effectively nullifies any sympathetic braking action to permit maximal secretomotor firing rates and hyperstimulation of mucosal secretion.

Apart from submucous secretomotor neurons, a major component of sympathetic input to the intestinal tract acts at presynaptic terminals to prevent release of excitatory transmitter substances that mediate fast or slow transmission at synapses within the integrated circuits of the enteric nervous system (see above section on noradrenergic presynaptic inhibition). These noradrenergic axo-axonal synapses function to inactivate the excitatory synaptic circuitry that mediates intestinal motor movements. The presynaptic action of inflammation-related mediators to suppress the sympathetic release of norepinephrine at synapses in the enteric nervous system is expected to prevent sympathetic inactivation of the enteric microcircuits that generate intestinal motor activity and other intestinal behaviors associated with the inflammatory state.

Responses of the Gut to Stress

Diarrhea and abdominal discomfort are common anecdotal descriptions of personal responses to emotional stress. As a professor of physiology, I am often asked by medical students to explain these symptoms as they are experienced in anticipation of a difficult examination. Activation of the sympathetic input to the bowel was used as an explanation in the past based on the blanket-like activation that occurs during "flight or fight" behavior. This explanation is ruled-out by consideration of the aspects of the neurophysiology at the interface of the sympathetic and enteric nervous systems discussed above. Activation of the sympathetic input in the absence of other factors (e.g., inflammation) suppresses mucosal secretion and therefore any tendency to diarrhea while simultaneously suppressing synaptic transmission in the circuits that initiate the propulsive motor events associated with abdominal discomfort. The current concept suggests that brain-mast cell interactions account for the manifestations of stress in the intestinal tract.

Enteric mast cells are believed to be involved in defense mechanisms that are separate from antigen sensing and the local signaling to the enteric nervous system described above (Fig. 7). The current hypothesis that mast cells are relay nodes for transmission of information from the brain to the enteric nervous system is plausible and of sufficient significance to justify attention. Evidence from ultrastructural and light microscopic studies suggest that enteric mast cells are innervated by projections from the central nervous system (48, 57, 18). Evidence supportive of the brain to mast cell connection is found in reports of Pavlovian conditioning of mast cell degranulation in the gastrointestinal tract (29). Release of mast cell protease into the systemic circulation is a marker for degranulation of enteric mucosal mast cells. This is found as a conditioned response in rats to either light or auditory stimuli and in humans as a conditioned response to stress (42). Findings that stimulation of neurons in the medulla oblongata by thyrotropin releasing hormone evokes degranulation of mucosal mast cells in the rat small intestine is additional evidence for brain-mast cell interactions (41). In the upper gastrointestinal tract of the rat, intracerebroventricular injection of thyrotropin releasing hormone evokes the same kinds of gastric inflammation and erosions as cold-restrain stress. In the large intestine, restraint stress exacerbates nociceptive responses and these effects are associated with increased release of histamine from enteric mast cells (20). Intracerebroventricular injection of corticotropin releasing factor mimics the response to stress. Injection of a corticotropin receptor antagonist or pretreatment with mast cell stabilizing drugs suppresses the stress-induced responses.

The brain to mast cell connection implies a mechanism linking central psycho-emotional status to irritable states of the digestive tract. The irritable state of the bowel with abdominal discomfort and diarrhea, which is know to result from degranulation of intestinal mast cells and release of signals to the enteric nervous system, is expected to occur irrespective of the mode of stimulation of the mast cells (Fig. 7). Degranulation and release of mediators evoked by neural input would have the same effect on intestinal motility and secretion as degranulation triggered by antigen detection. This could explain the similarity of bowel symptoms between those associated with noxious insults in the lumen and those associated with psychogenic stress in susceptible individuals.

Taken together, the evidence reinforces the hypothesis that moment-to-moment behavior of the

gut, whether it is normal or pathologic, is determined primarily by integrative functions programmed into the enteric nervous system. The enteric minibrain processes signals derived from local sensory receptors, the central nervous system (i.e., the parasympathetic and sympathetic divisions of the autonomic nervous system) and immune/inflammatory cells (e.g., mast cells). Enteric mast cells utilize the capacity of the immune system for detection of new antigens and long-term memory that permits recognition of the antigen if it ever reappears in the gut lumen. Should the antigen reappear, the mast cells signal its presence to the enteric minibrain. The minibrain interprets the mast cell signal as a threat and "calls-up" from its library of programs secretory and propulsive motor behavior that is organized for quick and effective eradication of the threat. Operation of the program protects the integrity of the bowel, but at the expense of the side effects of abdominal distress and diarrhea. The same symptomatology is expected to result from activation of neural pathways that link psychologic states in the brain to the mast cells in the gut. The brain to mast cell connections are not yet fully identified anatomically; nevertheless, consideration of the neurophysiology at the interface of the sympathetic and enteric nervous systems would seem to rule-out activation of sympathetic pathways and release of norepinephrine as factors in the stress-related symptomology of diarrhea and associated abdominal discomfort.

Acknowledgement

Work on neurogastroenterology in my laboratory has been supported by National Institutes of Health research grants RO1 AM26742, RO1 AM16813, RO1 NS17363, RO1 DK37238, RO1 AA07123, RO1 DK39937, RO1 DK 46941 and a Research Career Development Award.

References

1. Andriantsitohaina, R. and Surprenant. A. Acetylcholine released from guinea-pig submucosal neurones dilates arterioles by releasing nitric oxide from endothelium. *J. Physiol. (Lond.)* 453: 493-502, 1992.
2. Baird, W.W. and Cutbert. A.W. Neuronal involvement in type 1 hypersensitivity reactions in gut epithelia. *Br. J. Pharmacol.* 92: 47-655, 1987.
3. Bartho, L., Holzer, P., and Lembeck. F. Sympathetic control of substance P releasing enteric neurones in the guinea pig ileum. *Neurosci. Lett.* 38: 291-296, 1983.
4. Bornstein, J.C., Costa, M. and Furness. J.B. FIntrinsic and extrinsic inhibitory synaptic inputs to submucous neurones of the guinea-pig small intestine. *J. Physiol. (Lond.)* 398: 371-390, 1988.
5. Bungardt, E., Vockert, E., Feifel, R., Moser, U., Tacke, R., Mutschler, E, Lambrecht, G. and Surprenant. A. Characterization of muscarinic receptors mediating vasodilation in guinea-pig ielum submucosal arterioles by the use of computer-assisted videomicroscopy. *Eur. J. Pharmacol.* 213: 53-61, 1992.
6. Castro, G.A. Gut immunophysiology; regulatory pathways within a common mucosal immune system. *News Physiol. Sci.* 4: 59-64, 1989.
7. Cooke, H.J. Role of the "little brain" in the gut in water and electrolyte homeostasis. *FASEB J. 3*: 127-138, 1989.
8. Cooke, H.J. and Reddix R A. Neural regulation of intestinal electrolyte transport. In: Physiology of the Gastrointestinal Track 3rd Edition, edited by L.R. Johnson, D.H. Alpers, J. Christensen, E.D. Jacobson and J.H. Walsh. New York, NY: Raven Press, 1994, pp. 2083-2132.
9. Costa, M. and Furness. J.B. The simultaneous demonstration of adrenergic fibers and enteric ganglion cells. *Histochemie* 5: 343-348, 1973.
10. Costa, M. and Furness. J.B. Storage, uptake and synthesis of catecholamines in the intrinsic adrenergic neurons in the proximal colon of the guinea-pig. Z. Zellforsch. *Mikrosk. Anat.* 120: 364-385, 1971.
11. Cowles, V.E. and Sarna. S.K. Effects of T. spiralis infection on intestinal motor activity in the fasted state. *Am. J. Physiol.* 259: G693-G701, 1990.
12. Cowles, V.E. and Sarna. S.K. Trichinella spiralis infection alters small bowel motor activity in the fed state. *Gastroenterology* 101: 664-669, 1991.
13. Del Tacca, M., Soldani, G., Selli, M. and Crema. A. Action of catecholamines on release of acetylcholine from human taenia coli. *Eur. J. Pharmacol.* 9: 80-84, 1970.
14. Evans, R.J., Jiang, M.M. and Surprenant. A. Morphological properties and projections of electrophysiologically characterized neurons in the guinea-pig submucosal plexus. *Neuroscience* 59:1093-1110, 1994.
15. Falck, B. Observations on the possibilities of the cellular localization of monoamines by a fluorescence method. *Acta. Physiol. Scand.* 56: 1-25, 1962.
16. Feher, E. and Vajda. J. Degeneration analysis of the extrinsic nerve elements of the small intestine. *Acta. Anat.* 87: 97- 103, 1974.
17. Frieling, T., Palmer, J.M., Cooke, H.J. and Wood. J.D. Neuroimmune communication in the submucous plexus of guinea pig colon after infection with Trichinella spiralis. *Gastroenterology* 107: 1602-1609, 1994.
18. Gottwald, T., Lhotak, S. and Stead. R. Effect of truncal vagotomy and capsaicin on mast cells and IgA-positive plasma cells in rat jejunal mucosa. *Neurogastroenterol. Mot.* 9: 25-33, 1997.
19. Grovier, W.C., Sugrue, M.F. and Shore. P.H. On the inability to produce supersensitivity of catecholamines in intestinal smooth muscle. *J. Pharmacol. Exp. Ther.* 165: 71-77, 1969.
20. Gue, M., C. Del Rio-Lacheze, H. Eutamene, V. Theodorou, J. Fioramonti, and L. Bueno. Stress-induced visceral hypersensitivity to rectal distension in rats: role of CRF and mast cells. *Neurogastroenterol. Mot.* 9: 271-279, 1997.
21. Hirst, G.D.S. and McKirdy. H.C. Presynaptic inhibition at a mammalian peripheral synapse. *Nature (Lond.)* 250: 430-431, 1974.
22. Hu, H.Z., Liu, S.M., Ren, J., Xia, Y. and Wood. J. D. Actions of prostaglandins on electrical and synaptic behavior of submucous plexus neurons in guinea-pig small intestine. *Gastroenterology* 116: 655A, 1999.
23. Jacobowitz, D. Histochemical studies of the autonomic innervation of the gut. *J. Pharmacol. Exp. Ther.* 149: 358-364, 1965.
24. Jiang, M.M. and Surprenant. A. Re-innervation of submucosal arterioles by myenteric neurones following extrinsic denervation. *J. Auto. Ner. Sys.* 37: 145-154, 1992.
25. Jonakait, G.M., Tamir, H., Gintzler, A.R. and Gershon. M.D. Release of [3H] serotonin and its binding protein from enteric neurons. *Brain Res.* 174: 55-69, 1979.
26. Knoll, J. and Vizi. E.S. Presynaptic inhibition of acetylcholine release by endogenous and exogenous noradrenaline at high rate of stimulation. *Br. J. Pharmacol.* 40: 400-412, 1970.

27. Krokhina, E.M. and Chuvil'Skaya. L. M. Neuronal composition of the intramural ganglia of the gastrointestinal tract. *Bull. Exp. Biol. Med.* 79: 330-333, 1975.
28. Kunze, W.A.A. and Furness. J.B. The enteric nervous system and regulation of intestinal motility. *Ann. Rev. Physiol.* 61: 117-142, 1999.
29. MacQueen, G., Marchall, J., Perdue, M., Siegel, S. and Bienenstock. J. Pavolvian conditioning of rat mucosal mast cells to secrete rat mast cell protease II. *Science* 243: 83-84, 1989.
30. Malmfors, T. Studies on adrenergic nerves. *Acta. Physiol. Scand.* 64: 1-93, 1965.
31. Manber, L. and Gershon. M.D. A reciprocal adrenergic- cholinergic axoaxonic synapse in the mammalian gut. *Am. J. Physiol.* 5:E738-E745, 1979.
32. Mao, Y.K., Wang, Y.F. and Daniel. E.E. Characterization of neurokinin type 1 receptor in canine small intestinal muscle. *Peptides* 17: 839-843, 1996.
33. Marks, B.H., Samoraski, T. and Webster. E.J. Radioautographic localization of norepinephrine H3 in the tissues of mice. *J. Pharmacol. Exp. Ther.* 138: 376-381, 1962.
34. Neild, T.O., Shen, K.Z. and Surprenant. A. Vasodilatation of arterioles by acetylcholine released from single neurones in the guinea-pig submucosal plexus. *J. Physiol. (Lond.)* 420: 247-265, 1990.
35. Nishi, S. and North. R.A. Intracellular recording from the myenteric plexus of the guinea-pig ileum. *J. Physiol. (Lond.)* 231: 471-491, 1973.
36. Norberg, K.A. and Hamberger. B. The sympathetic adrenergic neuron. Some characteristics revealed by histochemical studies of the intraneuronal distribution of the transmitter. *Acta. Physiol. Scand.* 63: 1-24, 1964.
37. North, R.A. and Surprenant. A. Inhibitory synaptic potentials resulting from alpha 2 adrenoceptor activation in guinea-pig submucous plexus neurones. *J. Physiol. (Lond.)* 358: 17-33, 1985.
38. Paton, W.D.M. and Vizi. E.S. The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea- pig ileum longitudinal muscle strip. *Br. J. Pharmacol.* 35: 10-28, 1969.
39. Perdue, M.H., Marshall, J. and Masson. S. Ion transport abnormalities in inflamed rat jejunum: involvement of mast cells and nerves. *Gastroenterology* 98: 561-567, 1990.
40. Phillips, S.F. Motility disorders of the colon. In: Textbool of Gastroenterology 2nd Ed., edited by T. Yamada . Philadelphia, PA: J. B. Lippincott, 1995, pp. 1856-1875.
41. Santos, J., Esteban, S., Mourelle, M., Antolin, M. and Malagelada. J.-R.Regulation of intestinal mast cells and luminal protein release by crebral thyrotropin-releasing hormone in rats. *Gastroenterology* 111: 1465-1473, 1996.
42. Santos, J., Saperas, E., Nogueiras, C. Mourelle, M., Antolin, M., Cadahia, A. and Malagelada, J.R. Release of mast cell mediators into the jejunum by cold pain stress in humans. *Gastroenterology* 114: 640-648, 1998.
43. Sarna, S.K., Otterson, M.F., Cowles, V.E., Sethi, A.K. and Telford. G.L. In vivo motor response to gut inflammation . In Effects of immune cells and inflammation on smooth muscle and enteric nerves S.M. Collins and W.J. Snape eds CRC Press, Boca Raton, 1991, pp. 181-195.
44. Schemann, M. and Wood. J.D. Synaptic behavior of myenteric neurones in the gastric corpus of the guinea-pig. *J. Physiol. (Lond.)* 417: 519-535, 1989.
45. Sharkey, K.A., Lomax, A.E.G., Bertrand, P.P. and Furness. J.B. Electrophysiology, shape, and chemistry of neurons that project from guinea pig colon to inferior mesenteric ganglia. *Gastroenterology* 115: 909-918, 1998.
46. Shen, K.Z., Barajas-Lopez, C. and Surprenant. A. Functional characterization of neuronal pre and postsynaptic alpha adrenoceptor subtypes in guinea-pig submucosal plexus. *Br. J. Pharmacol.* 101: 925-931, 1990.
47. Song, Z.M., Brookes, S.J. H. and Costa. M. Identification of myenteric neurons which project to the mucosa of the guinea- pig small intestine. *Neurosci. Lett.* 129: 294-298, 1991.
48. Stead, R.H., Tomioka, M., Quinonez, G., Simon, G.T., Felten, S.Y. and Bienenstock. J. Intestinal mucosal mast cells in normal and nematode-infected rat intestines are in intimate contact with peptidergic nerves. *Proc. Natl. Acad. Sci. USA* 84: 2975- 2979, 1987.
49. Surprenant, A. and North. R.A. Mechanism of synaptic inhibition by noradrenaline acting at alpha$_2$ adrenoceptors. *Proc. R. Soc. (Lond.)* 234: 85-114, 1988.
50. Szurszewski, J.H. and King. B.F. Physiology of prevertebral ganglia in mammals with special reference to inferior mesenteric ganglion. In: Handbook of Physiology, The Gastrointestinal System, Motility and Circulation. edited by J. D. Wood. Bethesda, MD: American Physiological Society, 1989, pp. 519-592.
51. Szurszewski, J.H. and Krier. J. Thoracic and lumbar sympathetic neural pathways to the gastrointestinal tract. In: Peripheral Neuropathy. Edited by P. J. Dyck, P. K. Thomas, E. H. Lambert, and R. Bunge. Philadelphia, PA: W.B. Saunders, 1983, pp. 265-284.
52. Tack, J.D. and Wood. J.D. Synaptic behavior in the myenteric plexus of the guinea-pig gastric antrum. *J. Physiol. (Lond.)* 445: 389-406, 1992.
53. Tamura, K. and Wood. J.D. Electrical and synaptic properties of myenteric plexus neurones in the terminal large intestine of the guinea-pig. *J. Physiol. (Lond.)* 415: 275-298, 1989.
54. Wang, Y.Z. and Cooke. H.J. H2 receptors mediate cyclical chloride secretion in guinea pig distal colon. *Am. J. Physiol.* 258: G887-G893, 1990.
55. Wang, Y.Z., Palmer, J.M. and Cooke. H. J. Neuro-immune regulation of colonic secretion in guinea pigs. *Am. J. Physiol.* 260: G307-G314, 1991.
56. Waterfield, A. and Kosterlitz. H.W. Release of acetylcholine from the myenteric plexus of the guinea pig ileum. In: Fourth International Symposium on Gastrointestinal Motility, edited by E.E. Daniel. Vancouver: Mitchell Press, 1974, pp. 660-666.
57. Williams, R.M., Berthoud, H.R. and Stead, R. H. Association between vagal afferent nerve fibers and mast cell in rat jejunal mucosa. *Gastroenterology* 108: A941, 1995.
58. Wood, J.D. Communication between minibrain in gut and enteric immune system. *News Physiol. Sci.* 6: 64-69, 1991.
59. Wood, J.D. Electrical and synaptic behavior of enteric neurons. In: Handbook of Physiology, The Gastrointestinal System, Motility and Circulation edited by J. D. Wood. Bethesda, Maryland: American Physiological Society, 1989, pp. 465-517.
60. Wood, J.D. Gastrointestinal neuroimmune interactions. In: Advances in the Innervation of the Gastrointestinal Tract, Edited by G. E. Holle, and J. D. Wood. Amsterdam: Elsevier Scientific Press, 1992, pp. 607-615.
61. Wood, J.D. Histamine signals in enteric neuroimmune interactions. In: Neuroimmunophysiology of Gastrointestinal Mucosa, edited by M. H. Perdue, K. E. Barrett, H. J. Cooke, and D. W. Powell. New York: Annals New York Academy Sciences, 1992, pp. 275-283.
62. Wood, J.D. Neuro-immunophysiolgy of colon function. *Pharmacology* 47 (suppl 1): 7-13, 1993.
63. Wood, J.D. Physiology of the enteric nervous system. In: Physiology of the Gastrointestinal Tract 3rd edition, edited by L.R. Johnson, D.H. Alpers, J. Christensen, E.D. Jacobson and J.H. Walsh. New York, NY: Raven Press, 1994, pp. 423-482.
64. Wood, J.D. and Mayer. C.J. Adrenergic inhibition of serotonin release from neurons in guinea-pig auerbach's plexus. *J. Neurophysiol.* 42: 594-603, 1979.
65. Xia, Y., Hu, H., Z. Liu, S., Ren, J., Zafirov, D.H. and Wood. J.D. IL-1(and IL-6 excite neurons and suppress nicotinic and noradrenergic neurotransmission in guinea pig enteric nervous system. *J. Clin. Invest.* 103: 1309-1316, 1999.
66. Xia, Y., Lu, G., Zafirov, D.H., Sarna, S.K. and Wood. J.D. Effects

of platelet-activating factor on electrical and synaptic behavior of neurons in the submucous plexus of guinea-pig small intestine. *Gastroenterology* 110: A782, 1996.

67. Xia, Y., Zafirov, D.H., Cooke, H.J. and Wood. J.D. Actions of tumor necrosis factor TNα on electrical and synaptic behavior in the submucous plexus of guinea-pig small intestine. *Gastroenterology* 108: A945, 1995.