

Gut-to-Brain Signaling: Sensory Mechanisms

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Introduction

Intrinsic and extrinsic sensory neurons provide information about visceral distension, which generally corresponds to the volume of luminal contents, the chemical composition and temperature of ingested material and its movement along the mucosal surface of the gut. This input generates signals that regulate intestinal motility, blood flow, secretion and absorption and is thus critical for normal digestion. Most of these stimuli, however, are processed within the enteric nervous system and are thus not perceived. Similarly, much of the sensory information carried by extrinsic afferents serves homeostatic functions and does not reach the brain areas involved in conscious sensation. If we perceive changes within the gastrointestinal tract, either as innocuous or painful

stimuli, our ability to discriminate the location and type (modality) of a given stimulus is poor. This is due to the low density of visceral innervation and the polymodal character of visceral afferents, which typically can be activated by several stimulus modalities. Afferent pathways converge within spinal cord and supraspinal areas, resulting in referral of visceral stimuli, especially painful stimuli, to somatic sites, such as the right shoulder in a patient with acute cholecystitis. Finally, intense visceral stimulation often triggers strong autonomic and emotional responses. In the following sections, we will summarize current understanding and emerging concepts related to visceral sensation, principally discomfort and pain.

As already discussed in Chapter 1, the anatomical basis of gastrointestinal innervation is quite complex,

Gastrointestinal tract sensory pathways

Intrinsic primary afferent neurons (IPANs)

- Located within the submucosal and myenteric plexuses.
- Activate enteric reflexes that regulate motility, secretion and blood flow.

Extrinsic primary afferent neurons (EPANs)

Vagal afferents:

- Activated by mechanical (low-intensity), thermal and chemical stimuli.
- Cell bodies in nodose ganglion and central terminals in brainstem nucleus tractus solitarius.
- Input to brainstem and higher centers that regulate

autonomic function are generally not perceived.

- Contribute to chemoreception and autonomic and emotional responses to painful stimuli.

Spinal afferents:

- Activated by low- and high-intensity mechanical stimuli.
- Cell bodies in dorsal root ganglia and central terminals in superficial dorsal horn of spinal cord.
- Generally polymodal (i.e. respond also to chemical and thermal stimuli).
- Convey information about painful stimuli.

with intrinsic primary afferent neurons in the submucosal and myenteric plexuses and a dual extrinsic primary afferent innervation. While we have gained significant insight into the structure and function of the sensory innervation of the gut, surprisingly little is known about interactions between extrinsic and intrinsic sensory pathways and the contributions of intrinsic afferents in conscious sensation.

Mechanosensation and the gastrointestinal tract

The volume of hollow viscera changes frequently due to the ingestion, propulsion and expulsion of the luminal contents. Filling of any compartment within the gastrointestinal tract may trigger conscious sensation and – if the intraluminal pressure exceeds a value of around 30 mm Hg – discomfort or even pain. Therefore, controlled distension of hollow viscera is an appropriate mechanical stimulus to study sensory mechanisms. Studies in human volunteers demonstrate that intraluminal pressures below 10 mm Hg typically elicit no or only vague sensations. When the pressure exceeds 30 mm Hg, the stimulus becomes unpleasant or painful. The quality of the sensation depends in part on the length of the balloon or bag used to distend the organ. Because of the low density of innervation, spatial summation plays an important role in sensations from the gut, explaining why high, very localized pressures along the gut are not normally associated with conscious sensation. Animal experiments with a variety of experimental approaches have allowed us to better define pathways and mechanisms mediating mechanical sensation in the gastrointestinal tract.

High- and low-threshold mechanoreceptors

By isolating a nerve, teasing it into small filaments and placing it on a recording electrode, it is possible to study the action potential firing of a single nerve fiber (axon). Distension of the esophagus activates vagal afferents, located within the muscle layer, as mucosal application of local anesthetics does not abolish this response. Studies in the esophagus and stomach have demonstrated that these vagal afferents appear to fall into a similar functional category: they have a low activation threshold and stimulus response functions that

encode intensities into the noxious range. Activation in response to muscle contraction (tension) or small volume changes (stretch) is certainly consistent with the role of the vagus nerve in regulating the normal function of the proximal gastrointestinal tract. Interestingly, two distinct populations of mechanoreceptive afferents can be identified in the spinal visceral afferent innervation. One group is activated by low-intensity stimuli, analogous to vagal afferents, whereas the second group, which comprises about 20–30% of the spinal afferents, responds to distending pressures exceeding 30 mm Hg. High-threshold mechanosensitive fibers have been found in spinal afferents innervating the stomach (Fig. 3.1), esophagus, gallbladder, urinary bladder, colon and uterus. The parallel between human data showing a pain threshold above 30 mm Hg intraluminal pressure and the functional characteristics of high-threshold fibers suggest that these high-threshold mechanoreceptors function as nociceptors and mediate acute pain in response to noxious mechanical distension.

To examine whether spinal or vagal pathways mediate information about noxious mechanical stimulation of the stomach, we studied behavioral changes in response to noxious gastric distension. As expected, noxious intensities of gastric distension led to cessation of the normal exploratory behavior, which persisted after vagotomy and was abolished by splanchnic nerve resection. Thus, consistent with the potential role of high-threshold mechanoreceptors as nociceptors, behavioral data demonstrate that spinal pathways primarily mediate painful mechanical stimuli.

Mucosal mechanoreceptors

The most common and subtle mechanical stimulus along the gut is due to movement of the luminal contents, which deforms the mucosa. While this information is not consciously perceived, recent studies provide important insight into sensory mechanisms within the gastrointestinal tract. Nerve activity can be recorded *in vitro* when a hollow viscus and its nerves are dissected and placed in an appropriately designed recording chamber. The lumen can be exposed and subjected to defined stimuli. Gentle stroking of the mucosa triggers a rapidly adapting barrage of action potentials in some fibers, whereas high-intensity probing or stretching activates other fibers. Mucosal mechanore-

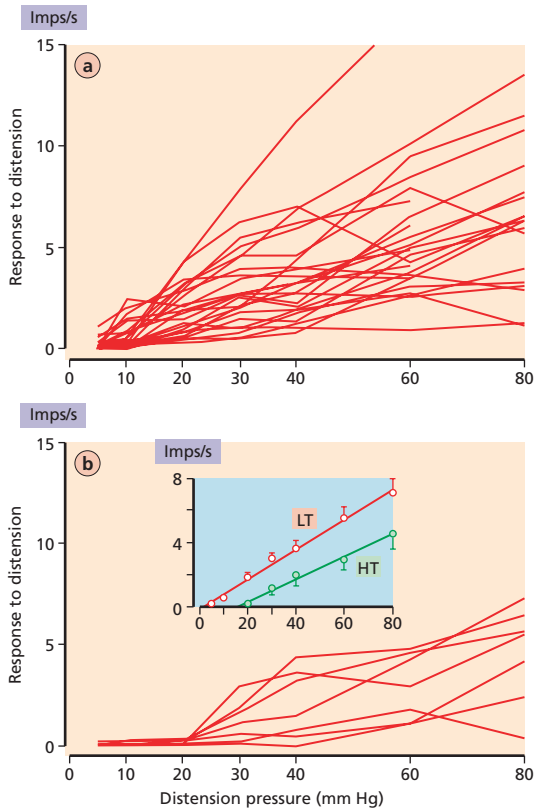


Fig. 3.1 Stimulus–response function of spinal afferents innervating the rat stomach. Whereas the majority of fibers are activated at distension pressures less than 10 mm Hg (a), a small population only responds to intragastric pressures exceeding 30 mm Hg (b). The insert summarizes the stimulus–response functions for low-threshold (LT) and high-threshold (HT) gastric splanchnic fibers. imps, impulses.

ceptors respond to very low-intensity, subtle mucosal stimuli and are potentially important in the regulation of blood flow, secretion or absorption. However, their physiological role is not fully understood.

Enteroendocrine cells, serotonin and mucosal mechanosensation

Stroking of the mucosa also triggers release of serotonin (5-HT) into the lamina propria of the epithelium and, to a lesser degree, the lumen of the gut. Serotonin is a monoamine neurotransmitter derived from the amino acid tryptophan. Most of the serotonin in the body is found within the gut, the specialized enteroendocrine

cells being the most abundant source. Intrinsic and extrinsic nerves within the gastrointestinal tract express 5-HT receptors, which in other experiments have been shown to be activated by 5-HT. Thus, 5-HT could function as one signal transmitting information about mucosal changes to afferent neurons. Consistent with this hypothesis, mechanical and chemical stimulation triggers 5-HT release from a cell line derived from human enteroendocrine cells. The most convincing evidence for a role of 5-HT in transducing mechanical stimulation comes from experiments demonstrating that mucosal stimulation does not elicit responses of submucosal neurons in the presence of 5-HT receptor blockers. The activation of these intrinsic primary afferent neurons by mucosally released 5-HT triggers enteric reflexes and alters secretion or muscle contraction. While a similar mechanism has not yet been directly shown for extrinsic afferents, 5-HT clearly affects vagal and spinal pathways and modulates gastric emptying, contributes to the gastrocolic reflex, and may be involved in triggering nausea. On the basis of these observations, several investigators have proposed a model according to which enteroendocrine cells and 5-HT play a pivotal role in the activation of intrinsic and possibly also extrinsic visceral afferents by mechanical and/or chemical stimulation of the gastrointestinal mucosa (Fig. 3.2). However, this concept needs to be extended to include other signaling molecules, as enteroendocrine and other epithelial cells release a variety of mediators that can affect nerve function.

Chemosensation and the gastrointestinal tract

The intestinal tract is continually exposed to changing luminal contents that contain different nutrients or even potentially noxious substances, such as high proton concentrations. While such alterations in luminal contents evoke neural responses and trigger changes in motility or secretion, we know relatively little about chemosensation within the gut. Duodenal infusion of nutrients or hypertonic saline relaxes the stomach and alters thresholds for discomfort and pain induced by gastric distension in healthy volunteers. When tested in animals, instillation of nutrients or hypertonic solutions into the duodenum activates vagal afferents. This may be due to direct activation of nerve endings located within

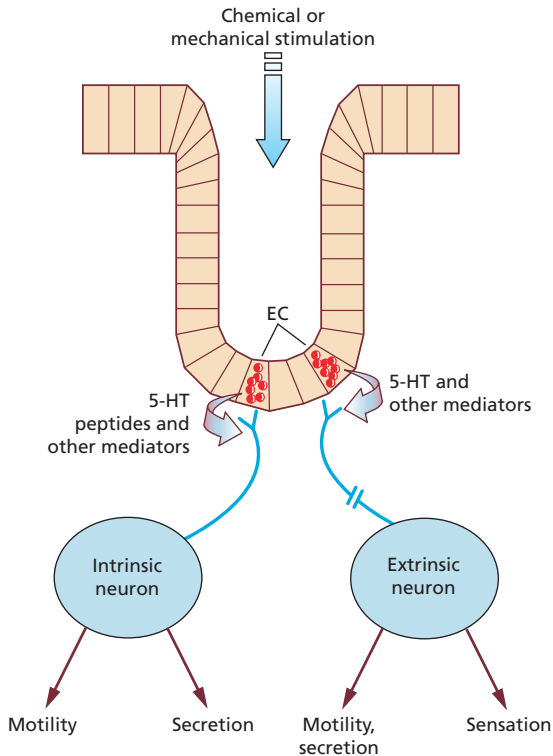


Fig. 3.2 Role of enteroendocrine cells and 5-HT in activating intrinsic and extrinsic sensory gastrointestinal neurons. Mucosal mechanical or chemical stimuli trigger release of 5-HT from enteroendocrine cells (EC) and other mediators from EC and other cells within the mucosa. The resulting increase in bioactive mediators in the lamina propria can activate intrinsic and extrinsic neurons, leading to changes in motility, secretion and sensation.

the mucosa or indirectly mediated by serotonin and/or other signaling molecules released by enteroendocrine or other cells. Using the single-fiber recording technique described above, acid-sensitive afferents have been identified in the esophagus, stomach and duodenum. In addition, by injecting a retrograde label into the intestinal wall, one can identify and selectively study the properties of the sensory neurons that innervate that area of the gastrointestinal tract. Using this approach, we recorded proton-gated currents in gastric neurons from nodose ganglia, the primary sensory ganglion of the vagus nerve, and T9–T10 dorsal root ganglia (Fig. 3.3), thus demonstrating that these neurons express ion channels that are directly activated by acid.

While vagal fibers are found within the mucosa, most spinal afferents terminate in the outer layers of the gut. The proximity of vagal endings to potentially noxious luminal stimuli raises the question of whether vagal fibers convey chemonociceptive information. Consistent with this hypothesis, instillation of high acid concentrations into the stomach activates only vagal and not spinal pathways (based on the expression of *c-Fos*, an immediate early gene that is transcribed after intense peripheral stimulation). The importance of the vagus is further supported by recent data showing that vagotomy, but not splanchnic nerve resection, abolishes the behavioral response to intragastric administration of acid. Thus, both spinal and vagal pathways are involved in gastric nociception. Accumulating evidence suggests that gastric spinal afferents convey mechanonociceptive information to the spinal cord, and that gastric vagal afferents convey chemonociceptive information to the brainstem and contribute to the autonomic and emotive response to noxious stimulation.

Specificity of gastrointestinal afferents

Appropriate discrimination of sensory information requires specific information about the location and modality of a given stimulus. Functional studies of gastrointestinal afferents have demonstrated that many fibers have more than one receptive field. Thus, peripheral visceral nerve terminals branch out and can collect information from different areas within a viscus, conveying the information along a single primary sensory neuron axon to the central nervous system. Importantly, the central terminations of visceral afferents typically diverge widely within the spinal cord, establishing synaptic contacts with several second-order neurons in different spinal segments. In addition, most visceral afferents are polymodal, i.e. respond to more than one stimulus modality, such as stretch and heat or chemical stimulation. This corresponds with clinical observations about the poor ability of patients to localize visceral pain and the unreliable discrimination of stimulus types, such as the sensation of heartburn during esophageal distension.

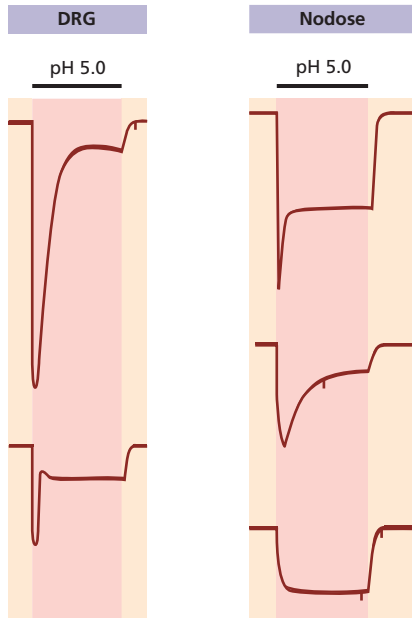


Fig. 3.3 Acid-sensitive ion currents recorded from gastric afferent neurons. Acid triggers transient inactivating inward currents in gastric dorsal root ganglion (DRG) sensory neurons (left). In contrast, only about half of the gastric nodose sensory neurons exhibit similar transient currents; the remaining half exhibit only a sustained current (right).

Visceral sensation and disease

Pain is a common symptom in patients with gastrointestinal diseases. Up to 25% of the adult population in the USA experiences abdominal discomfort or pain

within a year and about 5–15% seek medical help. In about half of the cases, no structural or biochemical abnormality can be identified with appropriate clinical tests, leading to the diagnosis of functional diseases such as non-cardiac chest pain, non-ulcer dyspepsia or irritable bowel syndrome. Interestingly, patients with such functional disorders of the gastrointestinal tract experience pain or discomfort at lower distension pressures than healthy individuals, suggesting that changes in mechanosensation may contribute to their problem. Similarly, many patients with typical reflux symptoms do not have signs of esophageal injury (non-erosive reflux disease), raising the question of whether chemosensation is altered and is at least in part responsible for their symptoms.

Considering the pain associated with acute or chronic inflammation of the gastrointestinal tract, most experimental approaches investigating the possible contribution of changes in visceral afferents to pain syndromes study the effects of inflammation or inflammatory mediators and cytokines.

Sensitization of visceral afferent pathways

Acute or chronic visceral pain typically decreases exploratory behavior and triggers aversive reactions in experimental animals. However, observation of such behavioral changes is variable and subjective, and has limited sensitivity in detecting changes in visceral sensation. A component of the aversive response – the contraction of abdominal wall or other muscle groups

Sensitization

Sensitization of *peripheral* visceral sensory neurons is defined by:

- increase in the number of action potentials triggered by a stimulus
- decrease in stimulus intensity required for action potential generation
- lowering of the threshold for action potential generation

Sensitization of *peripheral* visceral sensory neurons may be associated with:

- increase in transmitter release at central synapses
- change in transmitters released at central synapses
- enhanced response (increased excitability) of postsynaptic neurons

Sensitization of *central* visceral sensory neurons is associated with:

- increase in response magnitude of central neurons
- increase in size of area of referred sensation
- increased excitability of spinal *and* supraspinal neurons

– can be monitored and quantified by recording electromyographic (EMG) activity in anesthetized or awake animals. This visceromotor response is a supraspinal reflex mediated within the brainstem and persists after decerebration. Visceral distension typically triggers a progressive increase in EMG activity when intraluminal pressure exceeds 20 mm Hg. Inflammation shifts this stimulus–response curve to the left, consistent with the development of hypersensitivity (Fig. 3.4).

Interestingly, the enhanced response to mechanical stimulation can persist for up to 6 weeks, long after repair of the initial injury, suggesting that inflammation can cause lasting changes in visceral sensation. Such

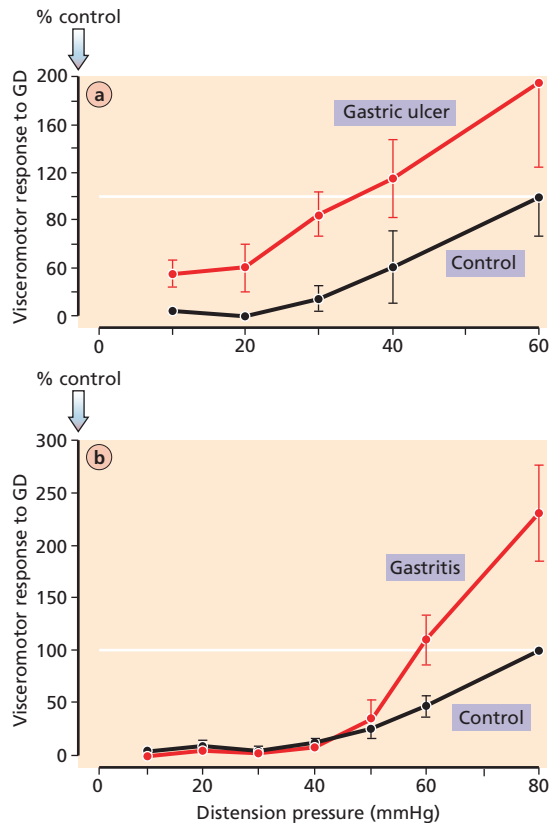


Fig. 3.4 Inflammation sensitizes responses to gastric distension (GD). The visceromotor response to gastric distension was quantified as EMG activity recorded from neck muscles in control animals and after induction of gastric ulcers (a) or mild gastritis (b). Inflammation shifted stimulus–response curves to the left, consistent with the development of gastric hypersensitivity.

changes may explain the persistence of symptoms after acute infections, such as postinfectious non-ulcer dyspepsia or irritable bowel syndrome. The hypersensitivity is not restricted to mechanical stimuli, as responses to acid are similarly enhanced.

Changes in the properties of extrinsic primary sensory neurons (*peripheral sensitization*) and processing centers at various levels within the spinal cord and/or brain (*central sensitization*) both play a role in the development of visceral hypersensitivity. Using different techniques, several investigators have studied the contribution of primary afferents. Recordings from single afferent fibers *in vivo* or *in vitro* demonstrate that sensory neurons can be acutely sensitized. Bradykinin, prostaglandin E₂, platelet-activating factor, histamine and other inflammatory mediators activate a subset of visceral afferents and in many cases enhance their response to subsequent mechanical stimulation (Fig. 3.5). Similarly, experimentally induced inflammation increases nerve responses to visceral distension or chemical stimulation.

Mechanisms of peripheral sensitization

On the cellular level, sensitization translates into increased excitability of a given afferent neuron; that is, lower stimulation intensity is needed to trigger an action potential, and/or a given stimulus triggers more action potentials. This is consistent with experimental results obtained in isolated neurons innervating the gastrointestinal tract. Stimulation of gastric sensory neurons with depolarizing current injections triggers action potentials. When cells obtained from animals with gastric ulcers are studied, the same stimulus elicits more action potentials (Fig. 3.6), reflecting an increase in neuron excitability.

Voltage-gated ion channels that are activated during depolarization form the basis of the action potential. The rapid upstroke is primarily due to the opening of sodium channels, with sodium influx into the cell and depolarization, while potassium channel opening causes potassium efflux and restoration of the negative membrane potential. Recent studies show that the expression of voltage-gated channels in visceral sensory neurons changes during inflammation. While sodium currents are increased and are more easily activated, potassium currents decrease, consistent with enhanced neuron excitability. Amitriptyline and κ -opioid ago-

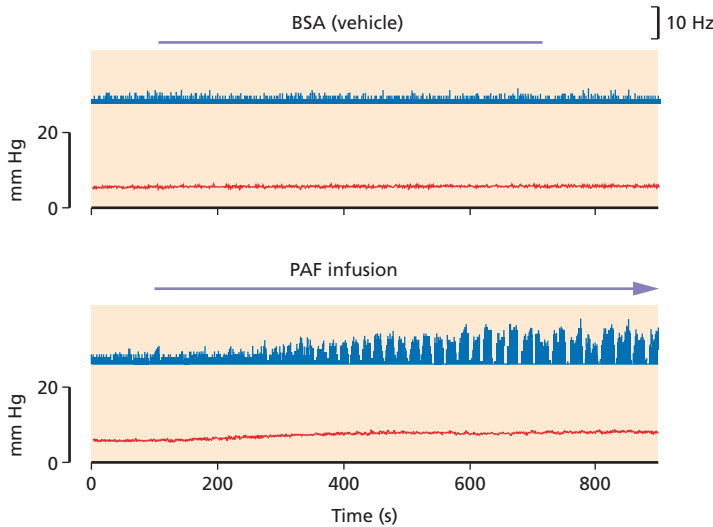


Fig. 3.5 Inflammatory mediators activate gastric vagal afferent fibers. While the vehicle did not affect the basal firing of mechanosensitive vagal afferent fibers, intra-arterial injection of platelet-activating factor (PAF) significantly increased firing.

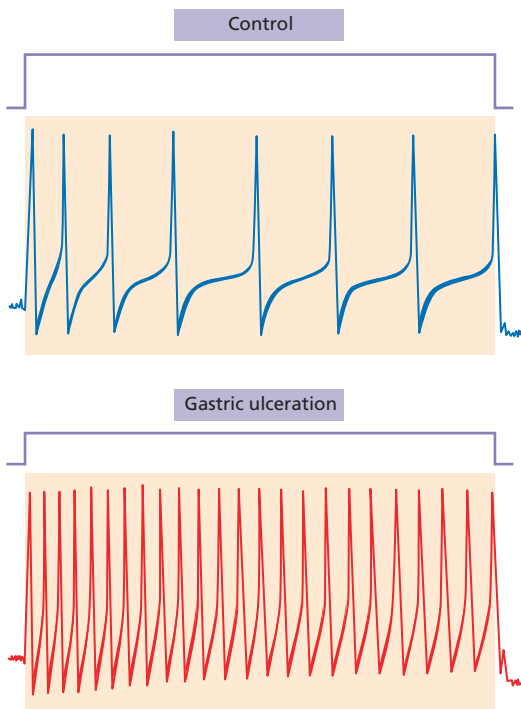


Fig. 3.6 Gastric ulcers increase the excitability of gastric sensory neurons. Depolarizing current injections trigger a short burst of action potentials in spinal gastric afferent neurons (control). When sensory neurons from animals with gastric ulcers were examined, the same stimulus intensity triggered significantly more action potentials (gastric ulceration).

nists have been used with some success in patients with functional bowel disorders. Interestingly, some κ -opioid agonists and tricyclic antidepressants use-dependently block voltage-gated sodium channels, which may contribute to their antinociceptive effects (Fig. 3.7). While these results suggest that these channels are interesting targets for pharmacological therapies, the lack of specific agents with low affinity for sodium channels in other areas, such as the central nervous system and the heart, currently limits its clinical application.

Multiple mechanisms probably lead to changes in neuron excitability during inflammation. Neurons express receptors for many inflammatory mediators, such as prostaglandins, histamine and serotonin, which can activate intracellular second-messenger cascades that in turn change the properties of ion channels (Fig. 3.8). In addition, some of these mediators and growth factors change the expression of ion channels and other proteins, thus leading to long-lasting alterations in neuron properties. One of these factors is nerve growth factor (NGF), which increases in gastrointestinal inflammation in humans and in animal models of inflammatory diseases. NGF regulates the expression of ion channels and causes functional changes that are similar to those seen during inflammation. Blocking the effects of NGF with neutralizing antibodies blunts the development of visceral hypersensitivity, further supporting the role of this mediator in the development of pain syndromes.

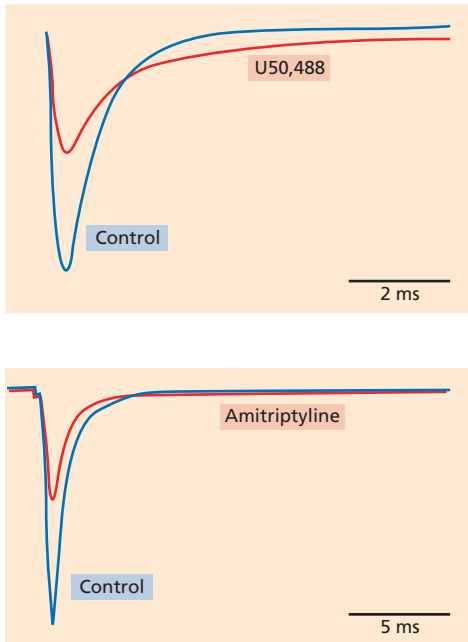


Fig. 3.7 Drugs used clinically affect inward, excitatory currents in visceral sensory neurons. Sample tracings show that the κ -opioid agonist U50,488 (10 μ M) and the tricyclic antidepressant amitriptyline (10 μ M) inhibit voltage-dependent sodium currents in visceral sensory neurons.

Serotonin and visceral hypersensitivity

As described above, 5-HT released from enteroendocrine cells plays an important role in activating sensory neurons. Mast cells are another potential source of 5-HT, as well as tryptase, histamine and other inflammatory mediators. Like enteroendocrine cells, mast cells are often found in close proximity to neurons. Enteroendocrine and mast cell numbers increase during inflammation and release more bioactive mediators. Interestingly, greater numbers of mast cells and enteroendocrine cells within the mucosa have also been reported in patients with functional bowel diseases. In experimental animals, inhibition of mast cell degranulation or administration of 5-HT receptor antagonists blunts responses to noxious visceral stimulation, supporting a possible role of this pathway in the sensitization of visceral afferents.

Despite these promising data, the contribution of 5-HT to human physiology and visceral pathophysiology is less clear. This is probably due to the multiple effects

of 5-HT on different serotonin receptors at different sites within the body, including smooth muscle, intrinsic nerves, extrinsic afferents and various processing sites within the central nervous system. Moreover, few 5-HT agonists and antagonists are available that selectively act on one member of the seven distinct families of serotonin receptors, all of which contain several subtypes. The reuptake through specialized transport systems is important in terminating the effects of 5-HT. Many antidepressant drugs interfere with this process by blocking the specific transporter. However, serotonin reuptake inhibitors do not consistently affect visceral sensation in humans. Alosetron, a selective 5-HT₃ receptor antagonist, blunts the response to noxious colorectal distension in rats. While this agent showed some promise in women with diarrhea-predominant irritable bowel syndrome, concern about drug-induced ischemic colitis led to temporary removal of this agent from the US market. Tegaserod, a 5-HT₄ receptor agonist, has recently been approved for the treatment of patients with constipation-predominant irritable bowel syndrome. It enhances the peristaltic reflex and accelerates colonic transit. While it also blunts visceral sensation, this effect may be indirectly mediated by changes in muscle tone.

Central modulation of visceral sensation

Information flow through visceral afferent pathways is modulated by inhibitory and facilitating influences from higher centers within the brain. Stress affects these modulatory circuits and may alter responses to visceral stimulation. Acute and chronic stress or stressful life events during periods with high neuronal plasticity, such as early postnatal life, enhance reactions to visceral distension in experimental animals. The similarly enhanced startle response points to hypervigilance (increased responsiveness to a variety of stimuli independent of their nature or location), which may also contribute to symptoms in patients with functional bowel disease.

Conclusion

Visceral distension and other stimuli activate afferent pathways that are important in the regulation of

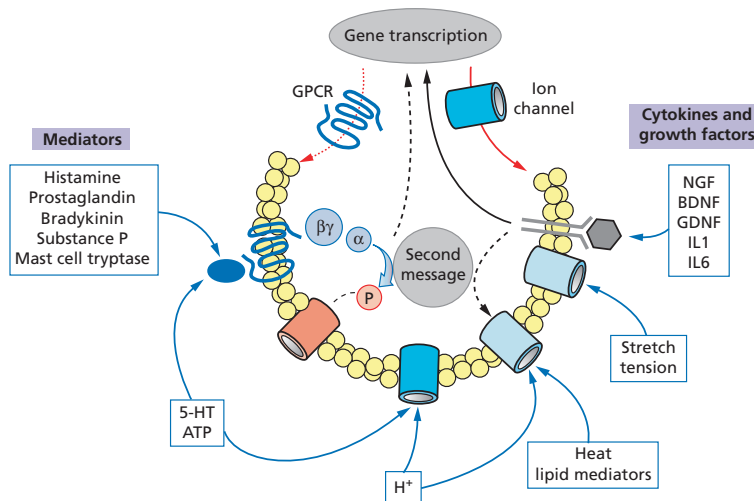


Fig. 3.8 Diagram of mechanisms of activation and sensitization of visceral sensory neurons. G protein-coupled receptors (GPCRs) and voltage/ligand-gated ion channels are synthesized in neurons and inserted into the cell membrane, where they are acted upon by extracellular and intracellular influences. Mediators such as bradykinin, substance P and serotonin (5-HT) act at bradykinin, neurokinin and 5-HT GPCRs (e.g. 5-HT₄ receptors), respectively. In consequence, intracellular coupling of the heterotrimeric $\beta\gamma$ and α G proteins activate second-messenger cascades that can lead to gene transcription and local activation of kinases and phosphorylation (P) of ion channels. For example, bradykinin phosphorylates the capsaicin

receptor ion channel TRPV1. Ion channels that gate Na⁺, K⁺ and Ca²⁺ ions are activated by ATP (e.g. P2X receptors) protons (H⁺), lipid mediators (e.g. HPETE), stretch/tension and 5-HT (e.g. 5-HT₃ receptors). Finally, cytokines and growth factors acting at their receptors can influence gene transcription as well as modulate the activity of other receptors. When tissue is insulted, mediators, growth factors and cytokines in the extracellular environment increase in concentration, activate their respective receptors and contribute to processes of sensitization, typically associated with an increase in action potentials generated by a stimulus and a reduction in stimulus intensity to generate an action potential.

normal intestinal function and provide the basis for conscious sensation of visceral phenomena and pain. Convergence of different stimulus modalities from more than a single receptive field onto a second-order neuron in the central nervous system and divergence of that sensory information at the level of the spinal cord and higher centers contribute to the poor localization and discrimination of visceral stimuli. Both vagal and spinal pathways contribute to unpleasant visceral sensations, such as the pain, fullness and discomfort experienced by patients with organic and functional diseases of the gastrointestinal tract. Peripheral processes, such as inflammation, and central modulatory pathways can alter the function of visceral afferent inputs and contribute to the development of visceral hypersensitivity.

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