Visceral Afferent Neurons: Role in Gastric Mucosal Protection

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Gastric mucosal homeostasis requires rapid alarm of protective mechanisms in the face of pending injury. This article summarizes the evidence that spinal afferent neurons monitor insults to the gastric mucosa and activate local mechanisms of defense and repair through release of transmitter peptides from their endings in the stomach.

Nowhere else in the digestive system is the mucosa more endangered than in the gastro-duodenal region, where it faces potential injury by noxious chemicals of endogenous and exogenous origin. Most tissues would rapidly disintegrate if exposed to the concentrations of HCl that bathe but do not harm the gastric surface epithelium. This is because a multitude of structural and physiological factors, collectively forming the gastric mucosal barrier, prevent hydrogen ions and other molecules from entering the tissue in quantities that produce cell damage. Although autonomic neurons have long been recognized to influence gastric mucosal defense against injury, the possibility that neurons constitute a rapid alarm system in the face of pending injury was hardly thought of. In particular, nociceptive afferent neurons that innervate the stomach and whose role it is to monitor tissue damage and in turn activate mechanisms of protection have been little considered until recently. This neglect of sensory neurons is understandable, though, if judged against the multiple innervation of the gut by intrinsic enteric neurons and extrinsic autonomic and afferent neurons, which has made a dissection of the specific roles played by afferent neurons very difficult.

Capsaicin as a probe for nociceptive neuron functions in the stomach

The discovery that sensory neurons contribute to gastric mucosal homeostasis resulted from exploitation of a neuropharmacological trait that differentiates them from other neurons (3). Many primary afferent neurons with C fibers and some with Aδ fibers are exclusively sensitive to the excitotoxic action of capsaicin, the pungent ingredient in red pepper, because they express vanilloid receptors of type 1 (VR1) that are specifically activated by capsaicin. As the vanilloid receptor-bearing afferent neurons are typically sensitive to noxious stimuli, capsaicin can be used to manipulate the activity of numerous, but not all, nociceptive afferents and thereby to probe their pathophysiological implications. Being an excitotoxin, capsaicin acutely stimulates and in the long term defunctionalizes nociceptive afferents including those innervating the gut, thus producing a chemical knockout of neurons that participate in the maintenance of gastric mucosal integrity.

Ablation of nociceptive neurons weakens gastric mucosal defense

The ability of nociceptive afferents to strengthen gastric mucosal resistance to injury first came to light when, at the International Congress of Physiological Sciences in 1980, Janós Szolcsányi and Loránd Barthó reported that chemical knockout of extrinsic afferent neurons aggravates experimental gastric injury. Since then, a large number of studies have confirmed and extended this pioneering observation (3). Pretreatment of rats with a neurotoxic dose of capsaicin does not cause damage by itself but exacerbates mucosal erosions caused by injurious factors such as stress, HCl, bile salt, acetylsalicylic acid (aspirin), indomethacin, and ethanol. The defensive potential of nociceptive afferents is further underlined by the finding that the ability of several substances and drugs to prevent gastric mucosal injury is compromised by neurotoxic doses of capsaicin. For instance, the gastroprotection afforded by prostaglandin E2, cholecystokinin, gastrin, the proton pump inhibitor lansoprazole, and the antacid hydrotalcit is reduced or abolished in capsaicin-pre-treated rats (3).
Stimulation of nociceptive neurons strengthens gastric mucosal defense

The inference of a gastroprotective role of nociceptive afferents, as deduced from the deleterious effect of capsaicin-induced neuron ablation, receives direct support from the observation that stimulation of these neurons strengthens gastric mucosal resistance to injury (3). Acute intragastric administration of excitatory doses of capsaicin attenuates the macroscopic and histological damage that HCl, bile salt, aspirin (Fig. 1), indomethacin, ethanol, and other injurious factors produce in the rat and canine stomach. The beneficial action of purified capsaicin on gastric mucosal homeostasis is shared by chili powder, which contains a large amount of capsaicin, and it is of particular importance to note that the gastroprotective effect of chili against aspirin-induced erosions is reproduced in humans. An epidemiological study does, in fact, suggest that dietary chili ingestion prevents peptic ulcer disease, because the incidence of ulcer disease in the population of Singapore correlates inversely with the amount of chili intake (7). In contradiction of traditional views attributing a gastric irritant action to capsaicin, this spice fails to alter back diffusion of acid, vascular permeability, and transmucosal potential difference and fails to cause histological damage to the gastric mucosa of rats and humans (3).

Pathways and mediators of neurogenic gastroprotection

The gastroprotective effect of capsaicin depends on an intact innervation by extrinsic afferents because it is prevented by chemical knockout of these neurons. It is evident, therefore, that capsaicin strengthens gastric mucosal defense by stimulating nociceptive afferents. The results of several neurochemical and pharmacological studies support the hypothesis that capsaicin-sensitive afferents afford gastric mucosal protection via release of calcitonin gene-related peptide (CGRP) and tachykinins such as neurokinin A (NKA) from their peripheral nerve endings (Fig. 2). However, the target cells, neural pathways (including extrinsic autonomic and intrinsic enteric neurons), and protective systems that are activated subsequent to this initial process have not yet been completely delineated (3). The case for a transmitter role of CGRP is particularly well advanced, given that in the rat stomach this peptide is exclusively expressed by extrinsic afferents, most of which originate from dorsal root ganglia of the caudal thoracic spinal cord (3). CGRP-containing axons are found in all layers of the stomach including the mucosa and form a particularly dense plexus around submucosal arterioles. Released by capsaicin from the peripheral terminals of spinal afferents, CGRP acts on CGRP1 receptors to enhance the resistance of the gastric mucosa to experimental injury. This pathophysiological role of the peptide...
has been proved with the use of the CGRP₁-receptor antagonist CGRP₈₋₃₇ and immunoneutralization of CGRP, both of which counteract the gastroprotective effect of capsaicin, and by the observation that active immunization of rats against CGRP exacerbates mucosal vulnerability by ethanol. NKA and related tachykinins, which are expressed in both extrinsic afferent and intrinsic enteric neurons of the stomach, also contribute to the neurogenic protection from ethanol-induced gastric damage through activation of tachykinin NK₂ receptors (13).

The gastroprotective action of the primary transmitters CGRP and NKA involves secondary messengers such as nitric oxide (NO), because the beneficial effects of capsaicin, CGRP, and NKA on the gastric mucosa are suppressed by NO synthase inhibitors (3, 13). NO is thus an important mediator of the gastroprotective pathways that are stimulated by capsaicin-evoked release of CGRP and NKA from extrinsic afferent nerve fibers (Fig. 2). A synergistic interaction between afferent neuron-derived peptides and NO can also be deduced from the effect of NO synthase inhibitors, which do not cause gastric acid damage in normal rats but lead to extensive gastric acid injury in rats in which capsaicin-sensitive afferents have been ablated (15). The source of NO involved in the antilesion actions of capsaicin, CGRP, and NKA has not been identified, so both a neural and an endothelial origin are conceivable. Some reports hold that inhibition of prostaglandin synthesis by indomethacin attenuates neurogenic gastroprotection, but, because capsaicin is unable to stimulate the formation of prostaglandin E₂ and I₂ (3), it would appear that prostaglandins contribute to activation of afferent neurons rather than postjunctional effects of protection.

**Mechanisms of neurogenic gastroprotection**

It was initially thought that the antilesion effect of capsaicin is directly related to the drug's ability to dilate submucosal arterioles and consequently increase gastric mucosal blood flow (6). This conjecture was corroborated when it was found that both hyperemia and gastroprotection are mediated by release of CGRP from afferent nerve fibers and subsequent formation of NO (6, 8, 15). The identity of mediators and the correlation between vasodilatation and attenuation of gastric injury suggested that hyperemia is the primary response that, in turn, supports defensive forces such as an appropriate delivery of bicarbonate to the surface mucus layer (3, 6).

It is now evident, however, that defensive mechanisms other than vasodilatation are also operated by nociceptive nerve fibers (Fig. 2). A dissociation of hyperemia and gastroprotection was clearly demonstrated when a NK₂-receptor antagonist was found to attenuate the antilesion effect of capsaicin in the rat gastric mucosa, whereas the concomitant hyperemia remained unaltered (13). This observation is in keeping with the ability of NK₂-receptor agonists to enhance gastric mucosal resistance to ethanol injury despite a marked reduction of mucosal blood flow. Importantly, a separation of hyperemia and gastroprotection has also been demonstrated for CGRP, which is able to attenuate gastric damage caused by endothelin-1 under conditions that preclude dilatation of the gastric microcirculation (3, 15).

The nature of the hyperemia-independent gastroprotective mechanisms operated by capsaicin-sensitive afferent neurons has not yet been identified with certainty, although many possibilities have been envisaged. One conjecture holds that CGRP may help maintain the integrity of the gastric mucosa by protecting the vascular endothelium from injury. Another argument goes that inhibition of gastric emptying, together with an increase in fluid secretion, results in dilution of injurious factors in the gastric juice and thus...
affords protection of the gastric mucosa (3). Under conditions in which hypersecretion of gastric acid contributes to injury, it is conceivable that the ability of CGRP to inhibit gastric acid output (9) attenuates gastric damage. Accumulation of acid in the gastric lumen induces nociceptive nerve fibers to release CGRP, which, via activation of CGRP$_1$ receptors, facilitates the release of somatostatin and depresses the release of gastrin, histamine, and acetylcholine and in this way inhibits further acid output (9). Other protective mechanisms are reflected by the ability of capsaicin to stimulate the secretion of mucus and bicarbonate in the gastroduodenal mucosa.

Neutral alarm system in the face of gastric acid back diffusion

The role of nociceptive afferents in the stomach is put into pathophysiological perspective if their function in response to acid challenge of the gastric mucosa is considered. Ingestion of alcohol, aspirin-like drugs, or irritant food is thought to focally disrupt the gastric mucosal barrier so that luminal acid and pepsin can intrude into the gastric mucosal tissue. This surge of back-fluxing acid is met with a prompt rise of blood flow through the gastric mucosa, a response that can be demonstrated experimentally when gastric acid back diffusion is induced by bile salt, ethanol, or hypertonic saline (3). The rapid signaling between the acid-threatened mucosal surface and submucosal arterioles is brought about by nociceptive nerve fibers, because the acid-evoked rise of gastric blood flow is blunted by chemical knockout of extrinsic afferent neurons (5). Inhibition of the hyperemia due to gastric acid back diffusion is associated with aggravation of mucosal damage, which attests to the defensive nature of the blood flow reaction. By facilitating the delivery of bicarbonate and the disposal of acid, hyperemia prevents the buildup of an injurious concentration of hydrogen ions in the tissue, supports other mechanisms of defense, and thus limits acid damage to the surface of the mucosa (3, 5).

The neural pathways and relays that underlie the gastric hyperemic response to acid back diffusion are not fully understood and differ in part from those of capsaicin-induced vasodilatation (Fig. 2). Studies involving surgical and pharmacological interventions have shown that the acid-evoked hyperemia arises from a peripheral reflex mechanism that relies on intact pathways in the splanchnic nerves and through the celiac ganglion (3). CGRP and NO are important mediators of the acid-evoked vasodilatation in the rat stomach (3), whereas endogenous tachykinins acting via NK$_2$ receptors dampen the gastric vasodilator response to acid back diffusion and thus appear to function as negative control factors (2).

Acid-monitoring afferents facilitate repair of the wounded mucosa

Although examined in less detail, it is increasingly evident that nociceptive afferents not only strengthen acute mucosal defense but also facilitate the repair of the injured mucosa. By monitoring acid influx into the gastric wall and increasing mucosal blood flow, these neurons support the process of restitution, which quickly restores the integrity of the superficial epithelium by mucous cell migration, and create favorable conditions for proper healing of the wounded mucosa (3). In addition, nociceptive neurons appear to promote per se repair processes, because the healing rate of gastric ulcers induced by HCl, acetic acid, or ethanol is delayed after chemical knockout of extrinsic afferents, whereas nociceptive neuron stimulation with low-dose capsaicin accelerates ulcer healing. The cellular mechanisms by which nociceptive afferents stimulate the repair of gastric ulcers remain to be determined.

Local alarm in the stomach is mediated by spinal afferents that do not signal to the brain

Because the neural emergency system in the stomach is constituted of nociceptive afferents, it is pertinent to ask whether the pathophysiological role of these neurons includes transmission of noxious information to the central nervous system and subsequent activation of autonomic and endocrine reactions. In considering this issue it must be realized that the stomach is innervated by two distinct groups of extrinsic afferents, spinal and vagal afferents (Fig. 3). The spinal afferents originate from cell bodies in the dorsal root ganglia and reach the stomach via the splanchnic and mesenteric nerves, whereas the afferent fibers in the vagus nerves have their cell bodies in the nodose and jugular ganglia. Nerve-selective ablation of extrinsic afferents has shown that the gastric hyperemia caused by intragastric capsaicin administration (8) and acid back diffusion (11) is largely brought about by spinal afferents passing through the celiac ganglion. This conclusion is consistent with the evidence that CGRP is the major transmitter of the neural alarm system in the gastric mucosa because, in the rat stomach, CGRP is almost exclusively present in spinal afferents.

Investigation of the afferent signaling of gastric acid challenge to the central nervous system has
revealed that the nociceptive neurons mediating local tissue homeostasis are distinct from those communicating the insult to the brain. Visualization of neuronal excitation via expression of the transcription factor c-fos shows that the gastric acid insult is signaled to the nucleus of the solitary tract and area postrema of the brain stem but not to the spinal cord (12). Because the afferent input to the brain stem is carried by vagal afferents, it would appear that spinal afferents projecting to the gastric mucosa are specialized in controlling local tissue homeostasis, whereas the central transmission of noxious information from the gastric lumen is conveyed by vagal afferents (Fig. 3).

Pathophysiological perspectives of the neural alarm system in the stomach

The spinal afferents that monitor insults to the gastric mucosa behave as a local emergency system because they appear to be silent under physiological conditions. Upon alarm, the fibers release CGRP as their major transmitter, engage NO as a secondary messenger, and thereby initiate reactions that strengthen the gastric mucosal defense and help repair the wounded mucosa (Fig. 2). Tachykinins also participate in the neurogenic protection from ethanol injury (13) but fail to enforce mucosal resistance to acid damage because they attenuate the protective rise of blood flow in response to acid back diffusion (2).

The pathophysiological significance of the alarm system comes to light if we consider the conditions under which the system is stimulated, the nature of the defensive reactions that in turn are triggered, and the pathological consequences that arise from a dysfunction of the system. Chemosensitive afferents respond to a wide range of chemicals that, besides acid and other injurious factors, comprise inflammatory mediators such as histamine, 5-hydroxytryptamine, bradykinin, and prostanoids as well as immunological messengers such as interleukin-1β (3). This spectrum of chemosensitivity enables capsaicin-sensitive afferent nerve fibers to detect potentially harmful changes in their environment and to initiate appropriate measures of defense and repair. In case of alarm, chemonociceptive afferents augment blood flow to the stomach and thereby aid the delivery of bicarbonate to the surface epithelium and overlying mucus layer, facilitate the removal of injurious factors from the mucosa, and thus promote defense and repair of the mucosa. This neural emergency system, which acts in concert with other mechanisms of protection, is operative not only in the stomach (3, 15) but also in other regions of the gastrointestinal tract (4).

The gastroprotective role of chemonociceptive afferents implies that dysfunction of the neural alarm system is likely to weaken gastric mucosal resistance to injury and thus may be an etiological factor in gastroduodenal ulcer disease. This conjecture receives experimental support from studies showing that sensory neuropathies disturb gastric mucosal homeostasis. For instance, chronic treatment of rats with oral taurocholate, to mimic chronic bile reflux, attenuates the capsaicin-evoked CGRP release and hyperemia in the gastric mucosa and weakens mucosal resistance to injury (10). Aging causes periarteriolar axons containing CGRP to disappear from the rat stomach, which is associated with a decline in the capsaicin- and acid-evoked increase in mucosal blood flow and an impairment of gastric mucosal defense (1). Sensory neuropathies arising from experimental diabetes also diminish CGRP release from nociceptive afferents in the rat stomach and lessen the ability of the gastric mucosa to defend itself against injury (14).

The concept that a derangement of nociceptive neuron function contributes to ulcer disease is in keeping with the emerging role of sensory neurons in functional bowel disorders. From a medical point of view, it is important to consider that drugs influencing the release, action, and metabolism of CGRP, NKA, and NO will interfere with the homeostatic function of peptidergic afferents in the stomach. For instance, opioid-receptor agonists such as morphine inhibit the release of peptide transmitters from afferent nerve fibers and in this way enhance the vulnerability of the gastric mucosa (3, 15). From a therapeutic perspective, it is obvious that future
strategies to manage ulcer disease will need to take into account measures that secure and strengthen the neural alarm system in the gastrointestinal mucosa.

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References