

P. HOLZER

### AFFERENT SIGNALLING OF GASTRIC ACID CHALLENGE

Department of Experimental and Clinical Pharmacology, Medical University of Graz, Austria

Gastric acid is a factor in the pain associated with peptic ulcer and other acid-related disorders including functional dyspepsia, given that antisecretory treatment is a mainstay in the treatment of upper abdominal pain. However, the molecular sensors, afferent pathways and central processing systems of gastric chemoreception are little known. This article reviews emerging evidence that vagal afferent pathways play a pivotal role in gastric chemoreception. Exposure of the rat gastric mucosa to backdiffusing concentrations of luminal acid is signalled to the brainstem, but not spinal cord, as visualized by functional neuroanatomy based on the rapid expression of c-fos. This observation is complemented by the finding that the visceromotor response to gastric acid challenge is suppressed by vagotomy, but not splanchnectomy. The gastric acid-induced expression of c-fos in the brainstem is reduced by inhibition of gastric acid secretion and enhanced by pentagastrin-evoked stimulation of gastric acid secretion. These data indicate that endogenous acid modulates the sensory gain of acid-sensitive vagal afferents. Further consistent with a role of these neurons in gastric nociception is the finding that exposure to proinflammatory cytokines and the induction of experimental gastritis or gastric ulceration sensitizes vagal afferent pathways to gastric acid. Taken together, these observations are of relevance to the understanding and treatment of gastric hyperalgesia and dyspeptic pain.

**Key words:** *Gastric acid; vagal afferent neurons; expression of c-fos in the nucleus tractus solitarius; visceromotor response; gastrin; gastric acid secretion; proinflammatory cytokines; gastric hypersensitivity; dyspeptic pain*  
*Contribution of gastric acid to upper abdominal pain*

Gastric acid is a factor in the pain associated with gastro-oesophageal reflux disease, gastritis and peptic ulcer (1 - 2). There is also mounting evidence that the painful symptoms of functional dyspepsia involve gastric acid as a noxious stimulus. The term dyspepsia refers to chronic or recurrent pain and discomfort

centered in the upper abdomen. If these prolonged symptom patterns occur in the absence of an identifiable organic cause, the disorder is classified as functional dyspepsia (3 - 5). Although the aetiology behind this frequent disease entity is largely unknown, it is obvious that afferent or central pain processing mechanisms play a role in the dyspeptic symptom profile. Conceptually, the pain of dyspeptic patients may reflect pathological alterations in gut function and/or signify that events in the gastrointestinal tract are represented in the brain in an exaggerated fashion because the sensory gain of afferent neurons or the central gain of afferent input from the gut is set abnormally high (6, 7). Besides sensitization of the gut-brain axis, summation of afferent inputs may also be of relevance to dyspeptic pain, given that unperceived electrical stimulation of mechano-insensitive jejunal afferents can increase the perception of background distension to an uncomfortable level (8).

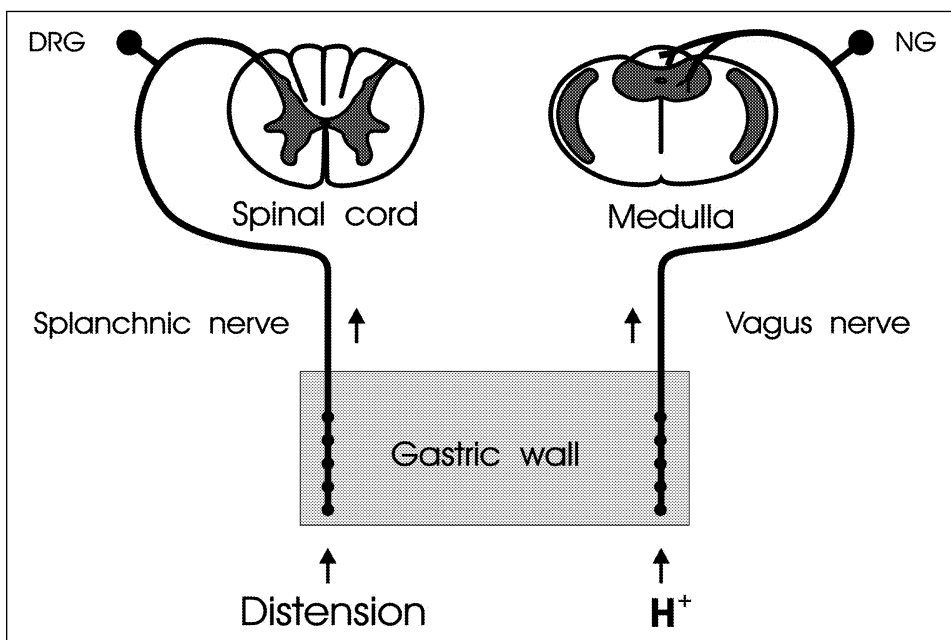
There is considerable evidence that patients suffering from functional dyspepsia are hypersensitive to mechanical stimuli such as gastric distension (9 - 12) as are patients with irritable bowel syndrome to colonic distension (3). The contribution of chemical stimuli has been less thoroughly investigated although there are reports that functional dyspepsia is associated with gastric hypersensitivity to fat and acid. For instance, lipid infusion into the duodenum contributes to the meal-like fullness and nausea which dyspeptic patients experience during gastric distension (13). Although gastric acid secretion is in the normal range (14), the stomach and duodenum of dyspeptic patients may be hypersensitive to acid (15, 16). In addition, acid can sensitize mechanosensitive afferents in the stomach (17). These observations and the ability of antisecretory therapy to alleviate symptoms in some dyspeptic patients (18) suggest that acid hypersensitivity, but not acid hypersecretion, is a factor in functional dyspepsia (19). Gastro-oesophageal reflux disease is likewise associated with an enhanced oesophageal sensitivity to acid, but not distension (20).

#### *Role of vagal afferents in gastric chemonociception*

Although it has long been thought that vagal afferents are not involved in abdominal pain, there is now growing awareness that these neurons make a distinct contribution to disease-related alterations in visceral sensation (21 - 23). The participation of vagal sensory neurons in nausea and emesis (24) and in cytokine-evoked illness responses (25, 26) corroborates the view that vagal afferents may determine the emotional-affective, neuroendocrine and behavioural aspects of abdominal nociception (27). That the vagus nerve plays a major role in the communication between the gut and the brain is beyond doubt. Thus, the vast majority (80 - 90 %) of the vagal axons are afferent nerve fibers (6, 24), making the vagus nerve the largest visceral sensory nerve in the body (28). Vagal afferents seem to tonically deliver information from the alimentary canal to the brain, and this sensory input is thought to be relevant not only to the autonomic regulation

of GI function but also to the interpretation of external sensory inputs, attitude and behaviour (28). In this context, the afferent part of the vagus nerve has even been suggested to mediate the “sixth sense” (28).

An important question pursued in my laboratory relates to the role which vagal sensory neurons play in the afferent signalling of gastric acid noxae. Using functional neuroanatomy, my group has found that exposure of the rat gastric mucosa to minimally injurious concentrations of hydrochloric acid (0.15 - 0.5 M HCl) leads to rapid expression of c-fos mRNA and c-Fos protein in the nucleus tractus solitarii (NTS) of the brainstem, the central termination area of vagal sensory neurons, but not in the spinal cord (23, 29, 30). The medullary c-fos response to gastric acid challenge is suppressed by bilateral subdiaphragmatic vagotomy, which indicates that the chemonociceptive afferent input from the gastric mucosa to the NTS and area postrema of the brainstem is predominantly carried by vagal afferents (23, 29). This is consistent with the observation that the visceromotor response to gastric acid challenge is abolished by vagotomy but not splanchnectomy (22). Since, in contrast, the visceromotor response to gastric distension is blocked by splanchnectomy, but not vagotomy (22), it follows that gastric mechano- and chemonociception are mediated by two different afferent pathways, vagal sensory neurons playing an exclusive role in the response to noxious acid (*Fig. 1*).



*Fig. 1.* Functional dissociation between mechano- and chemonociception in the stomach as revealed by experimental studies in the rat. The graph is based on data reported in references 21, 22, 23 and 29.

A comparative analysis of the medullary c-Fos induction and gastric damage indicates that the afferent signalling of gastric acid challenge is not directly related to the formation of overt mucosal injury, since c-Fos expression in the NTS can be evoked by HCl concentrations (0.15 - 0.35 M) that do not induce any macroscopic lesions and cause little histological damage (29, 30). Because supraphysiological concentrations of intragastric HCl (0.15 M or higher) are required to induce c-Fos in the NTS, it is inferred that only a massive increase of the proton gradient across the acid-tight gastric mucosal barrier is able to drive sufficient protons into the lamina propria where they can excite vagal afferent nerve fibers either directly (31 - 33) or indirectly via neuroactive factors released in the tissue. This experimental approach thus models pathophysiological circumstances where vagal afferents are stimulated by backdiffusion of luminal acid through a leaky gastric mucosal barrier whose permeability may increase, e.g., following the intake of irritating food or liquid, infection, inflammation, stress and nonsteroidal antiinflammatory drug use.

The vagal afferent input from the acid-threatened stomach is processed in the medullary brainstem such that the information is passed on to the lateral parabrachial nucleus, the thalamic and hypothalamic paraventricular nuclei, the supraoptic nucleus, the central amygdala and the mediolateral habenula, these projection areas being visualized by c-fos mRNA expression (23). There is, however, no activation of the insular cortex, the major cerebral representation area of afferent input from the stomach. Provided that functional c-fos neuroanatomy produces a valid image of brain activity, it would seem that vagal afferent signalling of gastric acid challenge does not give rise to perception of pain but leads to activation of subcortical brain nuclei that are involved in emotional, behavioural, autonomic and neuroendocrine reactions to noxious stimuli (23). This contention, though, requires confirmation by other experimental approaches, e.g., real-time functional brain imaging.

Although vagal afferents in the stomach have long been known to discharge action potentials when their peripheral terminals are exposed to acid (31 - 33), the molecular sensors of H<sup>+</sup> ions remain unknown. Deviations from physiological values of the extracellular pH can be monitored by multiple acid sensors on afferent neurons (34). Acid-sensing ion channels (ASICs) are activated by moderate acidification whereas transient receptor potential (TRP) cation channels, notably TRPV1, are gated by severe acidosis. In contrast, ionotropic purinoceptor (P2X) ion channels, notably P2X<sub>2</sub>, and two-pore domain background K<sup>+</sup> channels, such as TASK, do not directly signal acidification but rather modulate cell membrane excitability in response to acidosis (34). Since the acid-evoked afferent signalling is not altered by capsaicin pretreatment (29) it appears improbable that signal transduction in the acid-threatened stomach is solely accomplished by the capsaicin-sensitive TRPV1 (34).

Apart from a direct action of protons on vagal afferents, backdiffusing acid may stimulate sensory neurons by releasing mediators in the tissue that have an

excitatory action on afferent nerve fibres. Such a messenger could be 5-hydroxytryptamine (5-HT), because vagal afferents express 5-HT<sub>3</sub> receptors (33) and considerable quantities of 5-HT are released from the acid-injured mucosa (35), most probably from enterochromaffin cells but possibly also from platelets and mucosal mast cells. A contribution by 5-HT to the sensory neuron-stimulating effect of gastric acid backdiffusion needs to be proven, though, because this amine does not seem to contribute to the acid-evoked excitation of mesenteric vagal afferents (33). It also remains to be explored whether cytokines which can be induced by acid injury in the gastric mucosa (36, 37) play a mediator role.

#### *Regulation of vagal acid sensitivity by gastric acid secretion*

Clinical observations of the algescic effect of gastric acid (1, 2, 15, 16) and the ability of antisecretory treatment to alleviate dyspeptic symptoms (18) attest to the implication of gastric acid in nociceptive processes of the upper gastrointestinal tract. However, the relationship between endogenous gastric acid secretion and acid sensitivity of vagal afferents has not yet been thoroughly explored. Experiments in my laboratory have shown that inhibition of gastric acid secretion by cimetidine or omeprazole attenuates the ability of backdiffusing luminal acid to induce c-Fos expression in the NTS of the rat brainstem (30). Conversely, stimulation of gastric acid secretion by pentagastrin enhances the induction of c-Fos in the NTS caused by luminal acid challenge, an effect that is also attenuated by omeprazole (30). These data could be explained by assuming that endogenously secreted acid sensitizes vagal afferents to luminal HCl backdiffusion (30), much as luminal acid sensitizes mucosal mechanoreceptors (17). It remains to be explored, though, whether gastric acid secretion is at all maintained when supraphysiological HCl concentrations are present in the gastric lumen and how endogenously secreted acid reaches vagal afferent nerve endings in the lamina propria.

Further work has demonstrated that systemic pentagastrin, at a dose that maximally stimulates gastric acid secretion, is *per se* able to induce c-Fos in the NTS and area postrema, even in the presence of omeprazole (30). The NTS response to intraperitoneal pentagastrin is suppressed by the CCK<sub>1</sub> receptor antagonist dexloxiglumide and attenuated by the CCK<sub>2</sub> receptor antagonist itriglumide. In contrast, the pharmacology of the pentagastrin-evoked c-Fos response in the area postrema is different from that in the NTS because itriglumide is more effective than dexloxiglumide in blocking the c-Fos induction due to pentagastrin (30). From these observations it has been inferred that systemic pentagastrin activates both CCK<sub>1</sub> and CCK<sub>2</sub> receptors which are associated with two different pathways projecting to the NTS (30). The acid-independent NTS reaction to peripheral pentagastrin is primarily brought about by stimulation of CCK<sub>1</sub> receptors on vagal afferents. A second pathway of pentagastrin-induced stimulation of the NTS involves the area postrema which, like other circumventricular organs, is exempt from the blood-brain barrier and

thus directly accessible to circulating peptides. As the area postrema contains many CCK<sub>2</sub> and some CCK<sub>1</sub> receptors (38) and the CCK-induced activation of area postrema neurons is mediated by CCK<sub>2</sub> receptors (39) it is inferred that IP administered pentagastrin can enter this brainstem region and induce c-Fos expression primarily *via* activation of CCK<sub>2</sub> receptors (30). Importantly, neurons in the area postrema issue outputs to the NTS and have been shown to facilitate the processing of vagal afferent input to the NTS (40, 41).

From this and related work it can be concluded that vagal afferents transmit both physiological stimuli (gastrin) and pathological events (backdiffusion of luminal HCl) from the stomach to the brainstem. These communication modalities interact because, firstly, acid secretion enhances afferent signaling of gastric acid backdiffusion and, secondly, gastrin activates NTS neurons through stimulation of CCK<sub>1</sub> receptors on vagal afferents and of CCK<sub>2</sub> receptors on area postrema neurons projecting to the NTS. It is very likely that the summation of vagal afferent inputs elicited by physiological and noxious stimuli may have an important bearing on dyspeptic pain, since unperceived electrical stimulation of mechano-*insensitive* jejunal afferents can increase the perception of background distension to an uncomfortable level (8). In conclusion, the implication of endogenous acid and circulating gastrin in gut-brain communication may be of relevance to the understanding of acid-related disorders and their symptoms.

#### *Cytokine-evoked sensitization of the vagal gut-brain axis to acid*

A widely accepted hypothesis holds that the hyperalgesia associated with functional bowel disorders reflects hypersensitivity of the gut-brain axis, either at a peripheral or central level (3). It is, however, incompletely understood which pathological circumstances cause the sensory gain of the gut-brain axis to increase. Since gastroenteritis, which may have subsided long ago, is thought to be a risk factor for functional dyspepsia, irritable bowel syndrome and the associated discomfort and pain, it is thought that immunological and inflammatory processes initiate long-lasting changes in bowel function and afferent neuron sensitivity (42 - 45). If vagal afferents were to play a role in upper abdominal hyperalgesia, the question arises as to whether they can be sensitized under conditions of infection, inflammation and immune challenge. There is increasing evidence that this is indeed the case (22). Thus, acetic acid-induced ulceration of the rat stomach increases the excitability of nodose and dorsal root ganglion neurons, an effect that is related to an enhancement of tetrodotoxin-resistant sodium currents (46) and a reduction of A-type potassium currents (47). These alterations may, at least in part, be mediated by nerve growth factor whose formation is increased in the ulcerated stomach (48).

The increase in sensory neuron excitability following gastric inflammation or injury is borne out by a sensitization to noxious chemicals as demonstrated by an enhancement of the visceromotor response to gastric acid challenge in rats with

acetic acid-induced ulcers or iodoacetamide-induced gastritis (22). My group has obtained experimental evidence that proinflammatory cytokines can sensitize vagal afferent pathways to gastric acid challenge. Thus, systemic administration of interleukin-1 $\beta$  and tumour necrosis factor- $\alpha$  leads to an increase in the gastric acid-evoked expression of c-Fos in the brainstem (49). This state of hypersensitivity is maintained for a period of more than 2 days.

The ability of cytokines to sensitize vagal afferent pathways to gastric acid (49) is in keeping with the implication of vagal afferents in the communication between the peripheral immune system and the brain (6, 25, 26). There are several sites at which peripheral cytokines can interact with vagal afferent nerve fibres. Following endotoxin exposure, Kupffer cells (macrophage-like cells to screen blood and lymph) in the liver release interleukin-1 $\beta$  and thereby lead to the excitation of afferents in the hepatic branch of the vagus nerve (50). Furthermore, the abdominal vagus is associated with paraganglia and connective tissue containing macrophages and dendritic cells that respond to endotoxin challenge with synthesis of interleukin-1 $\beta$  (51, 52). The abdominal paraganglia of the vagus nerve contain glomus-like cells which have interleukin-1 receptors (53), are innervated by vagal afferents (54) and may hence serve as chemosensory accessory cells (25). In addition, vagal afferents innervate abdominal lymph nodes which represent another interface with the visceral immune system (25).

Electrophysiological recordings demonstrate that peripheral administration of interleukin-1 $\beta$  leads to increased firing in vagal afferents (55, 56) and induces c-Fos expression in the NTS (49, 57). Since interleukin-1 receptors of type I are expressed by nodose ganglion cells, it appears as if the cytokine excites vagal afferents by a direct action on the axons, although prostaglandins acting *via* EP<sub>3</sub> receptors and cholecystokinin acting *via* CCK<sub>1</sub> receptors may also contribute (56, 58). Moreover, interleukin-1 $\beta$  is able to increase the sensitivity of gastric vagal afferents to fire in response to PGE<sub>2</sub> and CCK (56, 59). Being responsive to peripheral interleukin-1 $\beta$ , vagal afferents participate in the behavioural *illness responses* to infection and inflammation, which comprise fever, anorexia, somnolence, decrease in locomotor activity, decrease in social exploration and hyperalgesia (25, 26). Accordingly, certain features of this *sickness behaviour* are attenuated by subdiaphragmatic vagotomy, although circulating pro-inflammatory cytokines can access the brain via circumventricular organs that are devoid of a blood-brain barrier (25, 26).

## CONCLUSIONS

In summary, the findings reviewed here provide strong evidence that vagal afferent neurons play a pivotal role in gastric chemonociception, particularly in the pain reactions to gastric acid challenge (*Fig. 1*). This important contribution of vagal afferents is corroborated by the observation that the acid sensitivity of

the vagal afferent system is upregulated by endogenous acid secretion, immune messengers, gastric inflammation and gastric ulceration. The fact that under these circumstances vagal afferent pathways are sensitized for a prolonged period of time makes a strong case for chemosensitive vagal nerve fibres being involved in the upper abdominal hyperalgesia associated with acid-related disorders including functional dyspepsia.

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Author's address: Peter Holzer, Ph.D., Professor, Department of Experimental and Clinical Pharmacology, Medical University of Graz, Universitätsplatz 4, A-8010 Graz, Austria.  
Tel: [+43] (316) 380-4500; Fax: [+43] (316) 380-9645  
E-mail: peter.holzer@uni-graz.at