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Acid sensing by visceral afferent neurons

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Abstract

Acidosis in the gastrointestinal tract can be both a physiological and pathological condition. While gastric acid serves digestion and protection from pathogens, pathological acidosis is associated with defective acid containment, inflammation and ischaemia. The pH in the oesophagus, stomach and intestine is surveyed by an elaborate network of acid-sensing mechanisms to maintain homeostasis. Deviations from physiological values of extracellular pH (7.4) are monitored by multiple acid sensors expressed by epithelial cells and sensory neurons. Protons evoke multiple currents in primary afferent neurons, which are carried by several acid-sensitive ion channels. Among these, acid-sensing ion channels (ASICs) and transient receptor potential (TRP) vanilloid-1 (TRPV1) ion channels have been most thoroughly studied. ASICs survey moderate decreases in extracellular pH whereas TRPV1 is activated only by severe acidosis resulting in pH values below 6. Other molecular acid sensors comprise TRPV4, TRPC4, TRPC5, TRPP2 (PKD2L1), epithelial Na^+ channels, two-pore domain K^+ (K_{2P}) channels, ionotropic purinoceptors (P2X), inward rectifier K^+ channels, voltage-activated K^+ channels, L-type Ca^{2+} channels and acid-sensitive G protein-coupled receptors. Most of these acid sensors are expressed by primary sensory neurons, although to different degrees and in various combinations. Since upregulation and overactivity of acid sensors appear to contribute to various forms of chronic inflammation and pain, acid-sensitive ion channels and receptors are also considered as targets for novel therapeutics.

Keywords

Acid surveillance; tissue protection; primary afferent neurons; acid-induced pain; acidosis; ischaemia; inflammation; acid-related gastrointestinal diseases; gastrointestinal tract; proton-gated currents; molecular acid sensors; acid-sensing ion channels; ASIC3; TRP ion channels; TRPV1; ionotropic purinoceptors

ACID SENSING BY SENSORY NEURONS

Acid as a stimulus for homeostatic mechanisms

Acid is one of the major secretory products of the stomach in which it serves to promote digestion and to protect the organism from pathogens ingested with food. At the same time, acidosis in the gastrointestinal tract can be a pathological condition that is caused by excess intake of acid, excess gastric acid secretion, defective acid containment, metabolic acidosis and acidosis due to ischaemia (hypoxia) or inflammation. To meet with these challenges, there are both cellular mechanisms of acid-base regulation and systemic monitoring systems

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to detect harmful acidosis, to trigger appropriate emergency reactions, and thereby to limit any tissue damage that may arise. Throughout the oesophagus, stomach and intestine, deviations from physiological values of extracellular pH (7.4) are monitored by multiple acid sensors expressed by epithelial cells and sensory neurons.

It has long been known that acid can elicit pain, and there is ample evidence that acidosis contributes to the pain associated with ischaemia and inflammation (Steen *et al.* 1992; Kress & Waldmann 2006; Wemmie *et al.* 2006). In the lumen of the stomach, gastric acid secretion causes the pH to drop to 1, and this acid load can only be managed by compartmentalization and a strong mucosal acid barrier in the foregut (Holzer 2007a). Intrusion of acid into the mucosa of the oesophagus, stomach or duodenum contributes not only to mucosal injury but also to the pain associated with gastro-oesophageal reflux and peptic ulcer disease (Texter1987; Huang & Hunt 1996; Holzer 2007b). However, peptic ulcer pain cannot be explained entirely by the effect of acid (Huang & Hunt 1996), and the author of this article speculates that the ulcer-related structural tissue damage may cause a sensory neuropathy that blunts acid-evoked pain.

Proton-gated currents in sensory neurons

Consistent with the ability of acidosis to induce pain is its capacity to excite primary sensory neurons and to sensitize them to other noxious stimuli (Holzer 2009). The molecular basis of acid sensing was discovered when proton-activated cationic currents were described in dorsal root ganglion (DRG) neurons (Krishtal & Pidoplichko 1981; Bevan & Yeats 1991). As reviewed by Kress & Waldmann (2006), two principal types of proton-gated inward currents are observed. The first type is characterized by a fast and rapidly inactivating inward current carried by Na^+ and by a high sensitivity to H^+, threshold activation occurring at a pH of 7 and maximum activation taking place at a pH around 6. While this type of proton-gated current is seen in most DRG neurons, the second type is observed only in DRG neurons that are also excited by capsaicin (Bevan and Geppetti 1994). Unlike the first type, this current is less sensitive to acidosis, activated only at pH levels below 6.2, sustained, slowly inactivating and developing tachyphylaxis on repeated activation (Petersen & LaMotte 1993). The sustained currrent is due to an increase in cation conductance that allows Na^+, K^+ and Ca^{2+} to pass (Zeilhofer *et al.* 1997).

Molecular analysis has shown that several acid-sensitive ion channels contribute to the capacity of afferent neurons to monitor acidosis (Holzer 2009). Among the molecular acid sensors, acid-sensing ion channels (ASICs) and transient receptor potential (TRP) vanilloid-1 (TRPV1) ion channels have been most thoroughly studied (Kress & Waldmann 2006; Wemmie *et al.* 2006; Szallasi *et al.* 2007). While the slow proton-activated conductance in DRG neurons shares many similarities with the acidosis-evoked current through TRPV1, the fast acid-induced current resembles currents carried by ASICs (Kress & Waldmann 2006). However, the characteristics of proton-gated currents in sensory neurons are complex, show regional and species differences and involve further acid-sensitive ion channels (Holzer 2009). This review first gives a brief overview of acid-sensitive receptors and ion channels that are expressed by primary afferent neurons and then goes on to review their implication in gastrointestinal physiology and pathophysiology.

ACID SENSORS OF SENSORY NEURONS

Acid-sensing ion channels (ASICs)

ASICs belong to the voltage-insensitive, amiloride-sensitive epithelial Na^+ channel/degenerin family of cation channels (Kellenberger & Schild 2002; Kress & Waldmann 2006). The *proton-sensitive* members of this family expressed in mammals are encoded by 3 different genes (*ACCN1*, *ACCN2* and *ACCN3*) which are alternatively spliced to produce 5

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subunits: ASIC1a, ASIC1b, ASIC2a, ASIC2b and ASIC3. These subunits are characterized by two membrane-spanning α-helical sequences (transmembrane domains 1 and 2), a large cysteine-rich extracellular loop and short intracellular N and C termini. The different subunits form distinct homomultimeric and heteromultimeric complexes, most likely of a tetrameric composition, which differ in their kinetics, external pH sensitivity, tissue distribution and pharmacological properties (Kress & Waldmann 2006; Wemmie *et al.* 2006). When activated, ASICs are preferentially permeable to Na^+ but some of them can also carry other cations such as Ca^{2+} (ASIC1a) and K^+ (ASIC1b).

The pH sensitivity of the ASIC subunits resides in several regions of the ASIC protein, particularly with His-72 and Gly-430 in the extracellular loop (Holzer 2009). ASIC1a, ASIC1b, ASIC2a and ASIC3 are directly gated by protons, whereas ASIC2b does not respond to acidosis when expressed as a homomultimer but can form functional heteromultimers with other ASIC subunits, particularly ASIC3. ASICs are activated by changes in pH only if they occur extracellularly, the threshold for activation of ASIC3 being as low as a fall of pH to 7.2 (Kress & Waldmann 2006; Wemmie *et al.* 2006). Although ASIC currents are in general fast and rapidly inactivating, there is evidence that they can also monitor prolonged acidosis. ASIC3 homomultimers and ASIC2a/ASIC3 as well as ASIC2b/ASIC3 heteromultimers produce two types of a sustained current: (i) a current that occurs at low acidic or even neutral pH and (ii) a current that is seen only at pH values below 5 (Kellenberger & Schild 2002; Kress & Waldmann 2006; Wemmie *et al.* 2006).

The properties of ASIC channel currents resemble the fast and rapidly inactivating inward Na^+ current that is evoked by minor acidosis in native DRG neurons (Krishtal & Pidoplichko 1981; Kellenberger & Schild 2002; Leffler *et al.* 2006). Further analysis revealed that proton-gated currents are mediated by ASIC1a or ASIC3 homomultimers in some instances and by ASIC2a/ASIC3 as well as ASIC2b/ASIC3 heteromultimers in other instances (Alvarez de la Rosa *et al.* 2002; Schicho *et al.* 2004; Kress & Waldmann 2006; Holzer 2009). There are distinct regional differences in the expression of ASIC1b, ASIC2b and ASIC3 which have been localized to spinal and vagal afferent neurons of small, medium and large diameter innervating the gut and other visceral organs (Kress & Waldmann 2006; Wemmie *et al.* 2006; Holzer 2007a). Retrograde tracing studies indicate that 75 % of the nodose ganglion neurons and 82 % of the DRG neurons projecting to the rat stomach express ASIC3-like immunoreactivity (Schicho *et al.* 2004). In mouse thoracolumbar DRGs, ASIC3 is expressed in 73 %, ASIC2 in 47 % and ASIC1 in 30 % of the somata projecting to the mouse colon (Hughes *et al.* 2007).

ASICs are related to ionotropic purinoceptors (P2X), some of which respond to extracellular pH changes (Kress & Waldmann 2006), as well as to the epithelial Na^+ channel δ subunit which seems to be an acid sensor in the human oesophagus (Yamamura *et al.* 2008).

Transient receptor potential (TRP) ion channels

The transient receptor potential (TRP) ion channels are named after the role these channels have in *Drosophila* phototransduction. At least 28 different TRP subunit genes have been identified in mammals (Clapham *et al.* 2005), comprising 6 subfamilies of the mammalian TRP superfamily. The primary structure of the TRP channels consists of 6 transmembrane domains with a pore domain between transmembrane domains 5 and 6 and with both the C and N termini located intracellularly (Clapham *et al.* 2005). Of the many TRP channel units, TRPV1, TRPV4, TRPC4, TRPC5 and TRPP2 are particularly sensitive to acidosis.

TRPV1—The existence of TRPV1 (initially termed vanilloid receptor 1, VR1), also known as the *capsaicin receptor*, has long been envisaged from the specific action of capsaicin on nociceptive afferent neurons (Jancsó 1960; Holzer 1991; Szallasi & Blumberg 1999). It is a

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polymodal nocisensor that is receptive not only to acid but also to noxious heat (above 43 °C), capsaicin and endovanilloids (Caterina & Julius 2001; Patapoutian *et al.* 2003; Holzer 2008). Assembled most likely as a homotetramer, TRPV1 is a non-selective cation channel with high permeability for Ca^{2+}. In addition, TRPV1 allows protons to enter the cell in an acidic environment (Hellwig *et al.* 2004; Vulcu *et al.* 2004). The conductance of H^+ through TRPV1 results in intracellular acidification, which in turn may act on membrane channels that are sensitive to changes in intracellular pH, e.g., certain two-pore domain K^+ channels.

The pH sensitivity of TRPV1 is fundamentally different from that of ASICs, because TRPV1 is gated open only if the extracellular pH is reduced below 6, in which case a sustained channel current is generated (Tominaga *et al.* 1998; Jordt *et al.* 2000). However, mild acidosis in the range of pH 7 - 6 can sensitize TRPV1 to other stimuli such as capsaicin and heat (Tominaga *et al.* 1998; Holzer 2008). As a result, the temperature threshold for TRPV1 activation is lowered under acidotic circumstances so that this cation channel becomes active at normal body temperature (Tominaga *et al.* 1998). Besides mild acidosis, many other signalling pathways (stimulated, e.g., by inflammatory mediators such as prostaglandins, bradykinin, adenosine triphosphate, 5-hydroxytryptamine and nerve growth factor) converge on TRPV1 and enhance the probability of channel gating by protons (Szallasi *et al.* 2007).

The ability of protons to sensitize TRPV1 to heat and other stimuli, on the one hand, and to activate TRPV1 *per se*, on the other hand, is mediated by different amino acid residues of the channel protein. Glu-600 on the extracellular side of transmembrane segment 5 is crucial for proton-induced sensitization of TRPV1, while Val-538 in the extracellular linker between transmembrane segments 3 and 4, Thr-633 in the pore helix and Glu-648 in the linker between the selectivity filter of the pore and transmembrane segment 6 are essential for proton-induced gating of TRPV1 (Jordt *et al.* 2000; Ryu *et al.* 2007). Mutation of these amino acid residues selectively abrogates proton-evoked currents but preserves the current responses to capsaicin and heat and their potentiation by mildly acidic pH (Jordt *et al.* 2000; Ryu *et al.* 2007). Thus, the sites in the TRPV1 protein targeted by protons differ from those targeted by other stimuli.

DRG neurons of TRPV1 null mice lack the slow and non-desensitizing proton-gated currents that are seen in DRG neurons of wildtype animals, whereas the fast and rapidly inactivating proton-gated currents mediated by ASICs are maintained (Caterina *et al.* 2000; Davis *et al.* 2000). The TRPV1-mediated currents due to acidification are largely confined to DRG neurons with unmyelinated fibres whereas the ASIC-mediated currents are also found on DRG neurons with myelinated axons (Leffler *et al.* 2006). This is consistent with the predominant expression of TRPV1 in unmyelinated primary afferent nerve fibres originating from the nodose and DRG ganglia (Holzer 2009). Of the nodose ganglion neurons that innervate the rat stomach, 42 - 80 % stain for TRPV1, whereas 71 - 82 % of the DRG neurons projecting to the rat stomach and mouse colon express TRPV1 (Patterson *et al.* 2003; Robinson *et al.* 2004; Schicho *et al.* 2004).

TRPV4—TRPV4 is gated by a drop of pH below 6 and the channel current reaches a maximum at a pH of about 4 (Suzuki *et al.* 2003). As TRPV4 has been localized to DRG neurons (Suzuki *et al.* 2003), a role of this TRP channel in acid sensing warrants exploration.

TRPC4 and TRPC5—The TRPC subfamily, specified as *canonical* or *classical*, is divided into three subgroups by sequence homology and functional similarities: C1/C4/C5, C3/C6/C7, and C2 (Clapham *et al.* 2005). Of these subunits, TRPC4 and TRPC5 respond to changes in extracellular pH, given that small decreases in pH (from 7.4 to 7.0) increase both

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G protein-activated and spontaneous TRPC5 currents (Semtner *et al.* 2007). TRPC4 channel activity is likewise potentiated by a decrease in pH. The effects of acidosis on TRPC4 and TRPC5 activity are biphasic, the currents being increased by a reduction of pH down to about 6.5 but inhibited when pH is decreased further (Semtner *et al.* 2007).

TRPP2 (PKD2L1, polycystic kidney disease-like ion channel)—PKD2L1 is a member of the polycystin (TRPP) subfamily of TRP channels and plays a major role in the sour taste (Huang *et al.* 2006; Ishimaru *et al.* 2006; LopezJimenez *et al.* 2006). In order to form functional channels, TRPP2 needs to associate as heteromer with related proteins of the PKD1 family (Ishimaru *et al.* 2006).

Two-pore domain K^+ (K_{2P}) channels

Two-pore (or tandem-pore) domain K^+ (K_{2P}) channels, encoded by the *KCNK* genes, represent one of the subfamilies of the large superfamily of K^+ channels. Defined by their membrane topology, these channels possess four transmembrane domains, two pore-forming loops between transmembrane domains 1 and 2 as well as 3 and 4, and a large extracellular linker region between transmembrane domain 1 and the first pore-forming loop (Goldstein *et al.* 2005; Duprat *et al.* 2007). Thus far, 15 human K_{2P} channel subunits have been identified and grouped into 6 structurally and functionally different subclasses, which are assembled as homo- or heterodimers (Goldstein *et al.* 2005; Duprat *et al.* 2007). Being primarily background channels, K_{2P} channels play a key role in setting the resting membrane potential as well as membrane input resistance and, consequently, the excitability of neurons. In addition, many K_{2P} channels are responsive to modifications of intra- and extracellular pH (Goldstein *et al.* 2005; Duprat *et al.* 2007; Holzer 2009). TASK-1, TASK-2, TASK-3, TRESK, TREK-1, TREK-2 and TRAAK are expressed by primary afferent neurons (Holzer 2009) any may contribute to the acid-sensing properties of these neurons. TASK channels are particularly receptive to variations in extracellular pH, given that TASK-1, TASK-2 and TASK-3 homo- and heteromers are inhibited by extracellular acidification (Goldstein *et al.* 2005; Duprat *et al.* 2007). Acid-induced inhibition of TASK channel activity will enhance nerve excitability and hence indirectly encode the presence of acid.

Proton-sensing G-protein-coupled receptors (GPCRs)

Certain G-protein-coupled receptors (GPCRs), notably OGR1, have turned out to be sensors of extracellular acidosis (Ludwig *et al.* 2003; Tomura *et al.* 2005). Like other GPCRs, these acid-sensitive receptors are composed of 7 transmembrane domains, their signalling involving G_s, G_i, G_q, and $G_{12/13}$ pathways. The sensitivity of OGR1 to extracellular pH changes resides with several histidine residues and is extremely high, given that half-maximum activation occurs at pH 7.2 - 7.5 and full activation at pH 6.4 - 6.8 (Ludwig *et al.* 2003; Tomura *et al.* 2005). The transcripts of proton-sensing GPCRs are widely distributed and, importantly, also expressed by DRG neurons, particularly by small-diameter afferent neurons that are involved in nociception (Huang *et al.* 2007).

Ionotropic purinoceptors (P2X)

P2X purinoceptors are ligand-gated membrane cation channels that open when extracellular adenosine triphosphate (ATP) is bound. They are assembled as homo- or heteromultimers (trimers or hexamers) of P2X subunits, seven of which ($P2X_1$ - $P2X_7$) have been identified at the gene and protein level (North 2002; Burnstock 2007). Their membrane topology is characterized by a very long extracellular loop between two transmembrane domains, with both the N and C termini located intracellularly (North 2002). Of the various P2X subunits, $P2X_1$, $P2X_2$, $P2X_3$, $P2X_4$, $P2X_5$ and $P2X_7$ are modulated by alterations in the extracellular

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pH (Holzer 2003). Thus, acidification reduces the potency of ATP to gate homomultimeric $P2X_1$, $P2X_3$, $P2X_4$ and $P2X_7$ receptors. In contrast, acidification sensitizes homomultimeric $P2X_2$ receptors to the excitatory effect of ATP (North 2002; Burnstock 2007). His-319 is particularly important for the effect of protons to potentiate the agonist effect of ATP on $P2X_2$, while protonation of His-206 and His-286 accounts for the inhibition of agonist-induced currents in $P2X_3$ and $P2X_4$, respectively (Gerevich *et al.* 2007; Holzer 2009). When different P2X subunits are coexpressed with each other, the resultant heteromultimers show a pH sensitivity that is different from that of P2X homomers (North 2002).

P2X receptors are expressed by many cells including primary afferent neurons. The P2X receptors on nodose ganglion neurons comprise predominantly homomultimeric $P2X_2$ and some heteromultimeric $P2X_{2/3}$ receptors whereas on DRG neurons homomultimeric $P2X_3$ prevail over heteromultimeric $P2X_{2/3}$ receptors (Burnstock 2007). Accordingly, the ATP-evoked inward currents in nodose ganglion neurons are persistent whereas those in DRG neurons exhibit transient, persistent or biphasic components (Dunn *et al.* 2001). Since only $P2X_2$ homomultimers and heteromultimers involving $P2X_2$, for instance $P2X_{2/3}$, are sensitized by acid, it is primarily $P2X_2$-containing purinoceptors that can function as indirect acid sensors. The monitoring of acidification depends on the concomitant release and/or presence of ATP or related purines whose agonist action is enhanced by a decrease of the extracellular pH. This scenario may be of pathophysiological significance, given that P2X receptors on afferent neurons are upregulated in inflammatory conditions such as inflammatory bowel disease (Yiangou *et al.* 2001a; Xu and Huang 2002).

Other acid-sensitive ion channels

Several members of the inward rectifier K^+ channel (Kir) family, such as Kir1.1, Kir4.1, Kir5.1 and Kir6.1 are highly sensitive to changes in the intra- or extracellular pH at near physiological levels (Holzer 2009). For instance, G-protein-coupled inward rectifier K^+ channels are activated by extracellular acidification (Mao *et al.* 2002). The inactivation of the voltage-activated K^+ channel $K_v1.3$ is delayed when extracellular pH is lowered, whereas the inactivation of $K_v1.4$ and and $K_v11.1$ channels is facilitated by extracellular acidosis (Jiang *et al.* 1999; Claydon *et al.* 2000; Somodi *et al.* 2004). Nifedipine-sensitive L-type Ca^{2+} channels can be activated by extracellular acidification, although there are also reports that high voltage-gated Ca^{2+} channels and tetrodotoxin-sensitive Na^+ channels are blocked by a drop of extracellular pH (Reeh & Kress 2001; Holzer 2009). Given that intracellular pH is regulated by ion pumps, it needs to be envisaged that many transporters including Na^+/H^+ exchangers can modify the acid sensitivity of chemosensory cells (Lyall *et al.* 2004; Montrose *et al.* 2006; Akiba & Kaunitz 2009).

PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL IMPLICATIONS OF ACID SENSORS IN THE GUT

Acidity and acidosis in the gut

The gastric parietal cells are the most productive source of acid in the body. These cells can secrete hydrochloric acid (HCl) to yield a H^+ concentration in the gastric lumen that - with an average diurnal pH of 1.5 - is 6 orders of magnitude higher than in the interstitial space of the gastric lamina propria (Holzer 2007a). While gastric acid is required for the conversion of pepsinogen to pepsin, contributes to the solubilization, digestion and absorption of several nutrients, and favours elimination of ingested pathogens (Pohl *et al.* 2008), the autoaggressive potential of HCl is kept in check by an elaborate network of mucosal defence mechanisms and by the functional compartmentalization of the oesophago-gastro-duodenal region (Holzer 2007b). Both strategies require an acid surveillance system among which acid-sensitive afferent neurons play an important role. When these surveillance systems fail,

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acid-related diseases including gastritis, gastroduodenal ulceration, dyspepsia and gastro-oesophageal reflux disease may ensue.

Acid sensors are not only relevant to control the secretion and actions of gastric acid but also to detect tissue acidosis resulting from ischaemia, inflammation, microbial activity, malignant tumour growth and gastrointestinal motor stasis. The pH profile in the gastrointestinal lumen of healthy subjects shows a distinct shape (Fallingborg 1999; Nugent *et al.* 2001), with peaks of acidity in the stomach and proximal large bowel. While HCl and bicarbonate (HCO_3^-) secretion are the major determinants of luminal pH in the foregut, luminal pH in the colon depends on mucosal HCO_3^- and lactate production as well as on microbial transformation of carbohydrates to short chain fatty acids and formation of ammonia. This pH profile can be changed by surgical interventions and in inflammatory bowel disease (Nugent *et al.* 2001; Holzer 2009).

Neurogenic inflammatory responses to acidosis

Acidosis-evoked stimulation of sensory neurons has two different effects: local release of neuropeptides from the peripheral nerve fibres in the tissue and induction of autonomic reflexes, sensation and pain (Holzer 1988; Holzer & Maggi 1998). By releasing peptide transmitters in the periphery, sensory nerve fibres can regulate vascular and other tissue activities, a process embodied in the term *neurogenic inflammation*. This efferent-like mode of operation may take place independently of nociception, and it has been hypothesized that some DRG neurons are specialized in controlling peripheral effector mechanisms only, while other DRG neurons may be specialized in the afferent mode of action or both (Holzer & Maggi 1998). The neuropeptides involved in the efferent-like mode of operation include calcitonin gene-related peptide (CGRP) and the tachykinins substance P and neurokinin A. Acidosis-evoked release of CGRP, one of the most potent vasodilator peptides, has been demonstrated in a variety of tissues including the gastric mucosa (Geppetti *et al.* 1991; Manela *et al.* 1995).

Acid sensing as a feedback mechanism in the control of foregut homeostasis

In order to avoid any tissue damage, the secretion of gastric acid must be tightly controlled according to need. The major inhibitory regulator is an increase in intragastric acidity, given that a decrease of luminal pH below 3 has a concentration-dependent inhibitory influence on HCl and gastrin secretion, and at pH 1 further acid output is abolished (Shulkes *et al.* 2006). The major mediator of this feedback inhibition is somatostatin which via paracrine and endocrine pathways inhibits parietal cell function both directly and indirectly via reduction of gastrin secretion. The activity of somatostatin-expressing D cells is in part regulated by acid-sensitive primary afferent neurons which following luminal acidification release CGRP to increase somatostatin secretion (Manela *et al.* 1995; Holzer 1998). The acid sensors of D cells and of other endocrine cells in the gastrointestinal mucosa await to be explored. Excess acid causes release of 5-hydroxytryptamine from enterochromaffin cells in the rat gastric mucosa (Wachter *et al.* 1998). Enterochromaffin cells are often called the “taste buds” of the gut, but whether they monitor intraluminal pH is not known.

When the oesophageal, gastric or duodenal mucosa is exposed to excess acid, there is a rapid activation of protective mechanisms including an increase in mucus gel thickness, HCO_3^- secretion and mucosal blood flow (Holzer 1998; Montrose *et al.* 2006; Akiba & Kaunitz 2009). These reactions are initiated in part by epithelial cells and their acid sensing mechanisms and in part by capsaicin-sensitive afferent neurons. Since the peripheral fibres of these neurons reside in the lamina propria behind the epithelium, the mucosal acid signal must be transduced across the epithelium. In the duodenum (Figure 1), this is achieved by diffusion of CO_2 into the epithelial cells, hydration to H^+ and HCO_3^-, intracellular

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acidification and exit of H^+ via the basolateral Na^+/H^+ exchanger of type 1 (Montrose *et al.* 2006; Holzer 2007b; Akiba & Kaunitz 2009). As a result, interstitial pH is lowered, a condition that activates sensory nerve terminals releasing the vasodilator peptide CGRP (Akiba *et al.* 2006b) which increases blood flow in part via a nitric oxide-dependent mechanism (Holzer 1998). TRPV1 is involved in the duodenal hyperaemia evoked by luminal acid exposure, since it is attenuated by the TRPV1 blocker capsazepine (Akiba *et al.* 2006b), whereas the gastric hyperaemia is left unaltered by capsazepine (Tashima *et al.* 2002).

Intraluminal CO_2 is a signal in the effect of acid to enhance both mucosal blood flow (Akiba *et al.* 2006b) and HCO_3^- secretion in the duodenum (Holm *et al.* 1998), but the acid-evoked output of HCO_3^- in the duodenum and stomach remains unchanged by capsazepine (Kagawa *et al.* 2003; Aihara *et al.* 2005). In addition, there is evidence that nitric oxide derived from salivary nitrate/nitrite contributes to some of the effects of luminal acid on the gastric mucosa. This applies both to the HCl -induced inhibition of peptone-stimulated gastrin release (Holm *et al.* 2000) and to the HCl-evoked increase in gastric mucus gel thickness and mucosal blood flow (Björne *et al.* 2004).

The mechanisms to keep the injurious potential of gastric acid in check involve not only adaptations of mucosal function and blood flow but also distinct motor effects causing compartmentalization of the oesophago-gastro-duodenal region (Figures 2 and 3). This strategy is to restrict the presence of high acid concentrations to the stomach, the mucosa of which is most resistant to intrusion by H^+, and to precisely control H^+ passage from the stomach to the duodenum through coordinated activity of the lower oesophageal and pyloric sphincters (Holzer 2007a, 2007b). Both sphincters are under the control of neural reflexes involving acid-sensitive neurons which adjust the tone of these sphincters to balance the levels of acid present in the oesophagus, stomach and duodenum with the mucosal defence mechanisms in these compartments (Forster *et al.* 1990; Lu & Owyang 1999; Holzer *et al.* 2003; Holzer 2007a). The molecular acid sensors and sensory neurons involved in the control of oesophago-gastro-duodenal motor activity await full identification (Holzer 2009). Apart from extrinsic sensory neurons, it is likely that intrinsic primary afferent neurons of the enteric nervous system are involved, given that they have been found to respond to acidosis (Bertrand *et al.* 1997; Schicho *et al.* 2003).

Paradoxically, knockout of TRPV1 has been reported to ameliorate acid-induced injury in the oesophagus and stomach (Akiba *et al.* 2006a; Fujino *et al.* 2006). Analysis of this unexpected observation in the stomach has revealed that disruption of the TRPV1 gene causes a compensatory upregulation of other protective mechanisms in the gastric mucosa (Akiba *et al.* 2006a). Thus, experiments with selective TRPV1 blockers are needed to unveil the precise role of TRPV1 in acid-induced mucosal injury in the foregut.

Acid-induced pain in the oesophagus and stomach

Gastric acid contributes to the symptoms and pain of gastro-oesophageal reflux disease and peptic ulcer (Kang & Yap 1991). Whether acid also plays a role in the pain associated with functional gastrointestinal disorders such as non-cardiac chest pain, functional dyspepsia, irritable bowel syndrome and functional abdominal pain syndrome is less well understood (Holzer 2007a). Whole-cell voltage-clamp recordings from DRG and nodose ganglion neurons innervating the rat stomach and mouse colon have shown that acidosis induces currents that can to a variable degree be attributed to the gating of ASICs and TRPV1 (Sugiura *et al.* 2005, 2007). The pH sensitivity and kinetics of these currents are distinctly altered after experimental induction of gastric ulcers (Sugiura *et al.* 2005).

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Exposure of the rat or mouse gastric lumen to supraphysiological HCl concentrations (>0.15 M) elicits a visceromotor response indicative of pain (Lamb *et al.* 2003) and causes many neurons in the nucleus of the solitary tract in the brainstem to express c-Fos, a marker of neuronal excitation (Schuligoi *et al.* 1998; Danzer *et al.* 2004; Wultsch *et al.* 2008). The gastric HCl-evoked visceromotor reaction and medullary c-Fos response are suppressed by vagotomy, but not transection of the sympathetic nerve supply to the stomach, which indicates that gastric HCl-evoked nociception depends critically on the integrity of the vagal afferent innervation (Schuligoi *et al.* 1998; Lamb *et al.* 2003). Apart from eliciting pain, acid causes sensitization of mechanosensitive afferent pathways from the oesophagus and stomach (Coffin *et al.* 2001; Medda *et al.* 2005). Experimentally induced gastritis and gastric ulceration enhance the gastric HCl-evoked visceromotor reaction and medullary c-Fos response (Lamb *et al.* 2003; Holzer *et al.* 2007; Wultsch *et al.* 2008).

The gastric HCl-evoked visceromotor reaction is inhibited by pretreatment of rats with a neurotoxic dose of capsaicin (Lamb *et al.* 2003). In contrast, the medullary c-Fos response to gastric acid challenge is not altered by pretreatment with capsaicin (Schuligoi *et al.* 1998). These discrepant findings might indicate that there are multiple acid sensing mechanisms in the oesophagus and stomach (Figure 3). Indeed, the stimulant effect of luminal acidification on gastric and oesophageal vagal afferent nerve fibres is attenuated in both TRPV1 null and ASIC3 null mice (Bielefeldt & Davis 2008). In addition, epithelial Na^+ channels containing the δ subunit appear to function as acid sensors in the human oesophagus (Yamamura *et al.* 2008). Examination of the brainstem c-Fos response to excess gastric acid revealed that afferent signalling from the normal stomach is preserved in ASIC3 knockout mice; however, the effect of gastritis to enhance the gastric acid-evoked expression of c-Fos in the brainstem is abolished by disruption of the ASIC3 gene (Wultsch *et al.* 2008). ASIC3 thus seems to play a major role in the inflammatory hyperresponsiveness of the vagal afferent - brainstem axis to gastric acid (Figure 3). Conversely, ASIC2 gene knockout does not alter inflammatory hyperresponsiveness but enhances the medullary c-Fos response to gastric acid challenge of the normal stomach (Wultsch *et al.* 2008). Although this finding suggests that ASIC2 may normally dampen acid-induced afferent input, it must not be forgotten that compensatory changes in germline knockout mice may obscure the functional implication of the disrupted gene.

Acid-induced pain in the small and large intestine

TRPV1 responds to acid and other noxious chemicals, and capsaicin-evoked activation of TRPV1 on intestinal afferent neurons elicits visceral pain in animals and humans (Drewes *et al.* 2003; Holzer 2004; Schmidt *et al.* 2004). Disruption of the TRPV1 gene and blockade of TRPV1 by capsazepine depress the acid-evoked stimulation of afferent nerve fibres supplying the mouse jejunum (Rong *et al.* 2004) and the acid-evoked currents in thoracolumbar and lumbosacral DRG neurons innervating the mouse colon (Sugiura *et al.* 2007). Experimental colitis induced by trinitrobenzene sulfonic acid is associated with an increase in TRPV1 expression in thoracolumbar and lumbosacral DRG neurons and in the visceromotor response to intracolonic acid administration (Figure 3). The effects of trinitrobenzene sulfonic acid to induce colitis, TRPV1 overexpression and hyperalgesia in response to acid challenge are counteracted by the TRPV1 blocker JYL1421 (Miranda *et al.* 2007).

There is only limited information about the role of P2X receptors in gastrointestinal sensation and pain. Protons potentiate the ATP-evoked stimulation of nodose ganglion and DRG cells (Dunn *et al.* 2001). Distension of the gut is thought to release ATP from the intestinal mucosa which subsequently excites afferent neurons expressing P2X receptors. This P2X receptor-dependent mode of mechanosensory transduction is enhanced by

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experimental colitis in the rat (Wynn *et al.* 2004) and may, conceivably, involve inflammation-associated acidosis.

Acid-induced pain in the peritoneal cavity

Intraperitoneal administration of acid causes behavioural manifestations of pain (Figure 3). Pharmacological blockade of TRPV1 with SDZ 249-665, a vanilloid compound causing desensitization of sensory neurons to capsaicin, attenuates the behavioural pain response to intraperitoneal administration of acetic acid in rats (Urban *et al.* 2000). This pain reaction may indeed reflect a response to acidosis because the writhing response to intraperitoneal injection of acetic, lactic and propionic acid is attenuated by capsazepine, whereas that to phenylbenzoquinone is not (Ikeda *et al.* 2001). An involvement of TRPV1 in acetic acid-induced writhing is further corroborated by the ability of various TRPV1 blockers to reduce the abdominal muscle contractions caused by intraperitoneal injection of acetic acid (Rigoni *et al.* 2003; Tang *et al.* 2007). Likewise, mice lacking TRPV4 are hyporesponsive to intraperitoneal injection of acetic acid (Suzuki *et al.* 2003). Overexpression of a dominant-negative ASIC3 subunit has been found to increase the writhing response to intraperitoneal acetic acid (Mogil *et al.* 2005), a change that paradoxically is also seen in ASIC3 null mice (Chen *et al.* 2002). Whether this finding is the result of a change in the kinetics of ASIC1 and ASIC2 after knockout of ASIC3 (Kress & Waldmann 2006) or of a compensatory upregulation of acid sensors other than ASIC3 is not known.

Apart from TRPV1 and ASIC3, P2X receptors also seem to be involved in peritoneal pain related to acidosis. The writhing behaviour elicited by intraperitoneal injection of acetic acid is inhibited by trinitrophenyl-ATP (a $P2X_1$, $P2X_3$ and $P2X_{2/3}$ receptor blocker) and A-317491 (a $P2X_3$ and $P2X_{2/3}$ receptor antagonist), whereas the $P2X_1$ channel blocker diinosine pentaphosphate is ineffective (Honore *et al.* 2002; Jarvis *et al.* 2002).

Upregulation of acid sensors in gastrointestinal disease

As reviewed above, there is emerging evidence that TRPV1 and ASIC3 play a role in acid sensing within the gastrointestinal tract as well as in the ulceration- and inflammation-evoked sensitization of afferent neurons. This inference is consistent with a number of findings that show that abdominal hyperalgesia is associated with an upregulation in acid sensor expression and/or function. For instance, acute exposure of the rat gastric mucosa to a noxious HCl concentration leads to a rise of TRPV1 immunoreactivity, but not TRPV1 mRNA, in DRG neurons innervating the stomach (Schicho *et al.* 2004). TRPV1 in vagal and spinal afferent neurons is upregulated in acid-evoked oesophagitis as well as in trinitrobenzene sulfonic acid-induced pancreatitis and colitis (Banerjee *et al.* 2007; Miranda *et al.* 2007; Xu *et al.* 2007). Similarly, the expression of TRPV1, ASIC3 and $P2X_3$ purinoceptors, but not ASIC1 and ASIC2, is enhanced in the colonic mucosa of patients with inflammatory bowel disease (Yiangou *et al.* 2001a, 2001b, 2001c). TRPV1-like immunoreactivity is likewise increased in oesophagitis (Matthews *et al.* 2004), non-erosive reflux disease (Bhat and Bielefeldt 2006), rectal hypersensitivity and faecal urgency (Chan *et al.* 2003).

PHARMACOLOGICAL MANIPULATION OF ACID SENSORS

Many of the acid-sensitive ion channels are highly regulated by endogenous factors and by therapeutic drugs. This is true, e.g., for nonsteroidal antiinflammatory drugs regulating the expression and function of ASICs (Holzer 2009), proinflammatory mediators causing sensitization of TRPV1 (Holzer 2008), and anaesthetics and other neuroactive drugs controlling the activity of K_{2P} channels. Moreover, several acid-sensitive ion channels and receptors including ASICs, TRPV1 and P2X are upregulated by inflammation and nerve

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injury and appear to contribute to the hyperalgesia associated with these conditions. If so, drugs targeting peripheral acid sensors may evolve as novel therapies for chronic inflammation and pain. This concept is attractive for a number of reasons, particularly because it offers the opportunity to develop antinociceptive drugs with a peripherally restricted site of action, avoiding unwanted effects on the central nervous system. However, interfering with molecular probes that are physiologically important poses a serious threat to homeostasis, unless selective inhibition of "excess" acid detectors can be achieved while their physiological function is preserved (Holzer 2009).

The challenge, therefore, is to elucidate differences in the number, location and molecular properties of physiologically relevant and abnormally active acid sensors and to pharmacologically differentiate between their physiological and pathological implications. Inflammation and chronic pain may be associated with activity-relevant polymorphisms in acid sensor genes, upregulation and/or ectopic expression of acid detectors, as well as overactivity and/or aberrations in the transduction mechanisms of acid sensors. As has been delineated for TRPV1 (Holzer 2008), there are several possibilities to focus therapy specifically on those acid detectors that contribute to the disease process. These approaches include (i) site-selective antagonists, (ii) uncompetitive (open channel) blockers that specifically silence overactive channels, (iii) drugs interfering with the sensitization (and desensitization) of acid detectors, (iv) drugs normalizing the overexpression of acid sensors by interfering with their intracellular trafficking, and (v) drugs that interfere with abnormal signal transduction of acid detectors.

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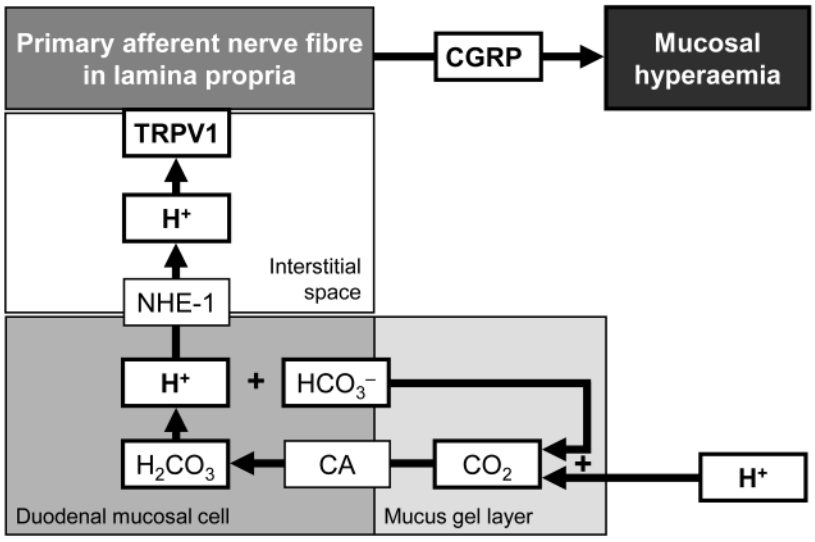

Figure 1.
Acid-sensing mechanisms underlying luminal acid-induced hyperaemia in the duodenum. CA, carbonic anhydrase; NHE-1, Na^+/H^+ exchanger of type 1.

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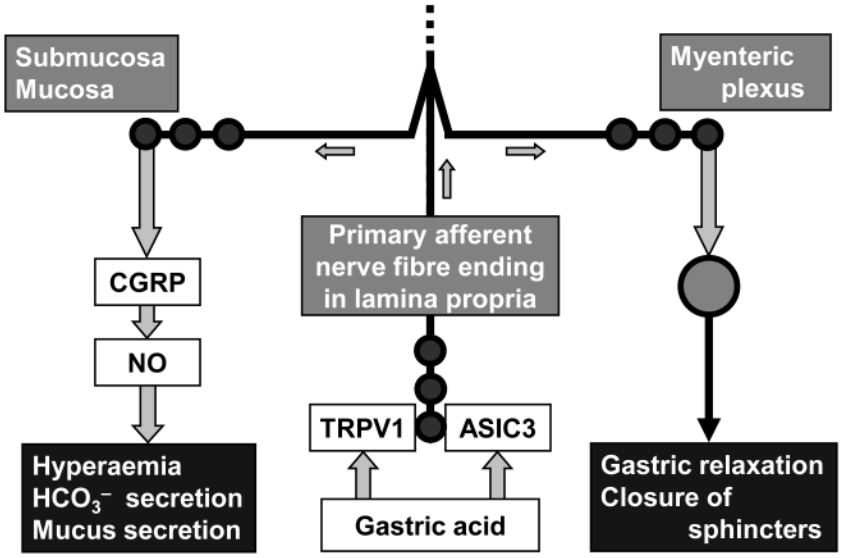

Figure 2.
Schematic diagram of mechanisms that keep the injurious potential of gastric acid in check. These mechanisms involve adaptations of mucosal function and blood flow as well as distinct motor effects causing compartmentalization of the oesophago-gastro-duodenal region.

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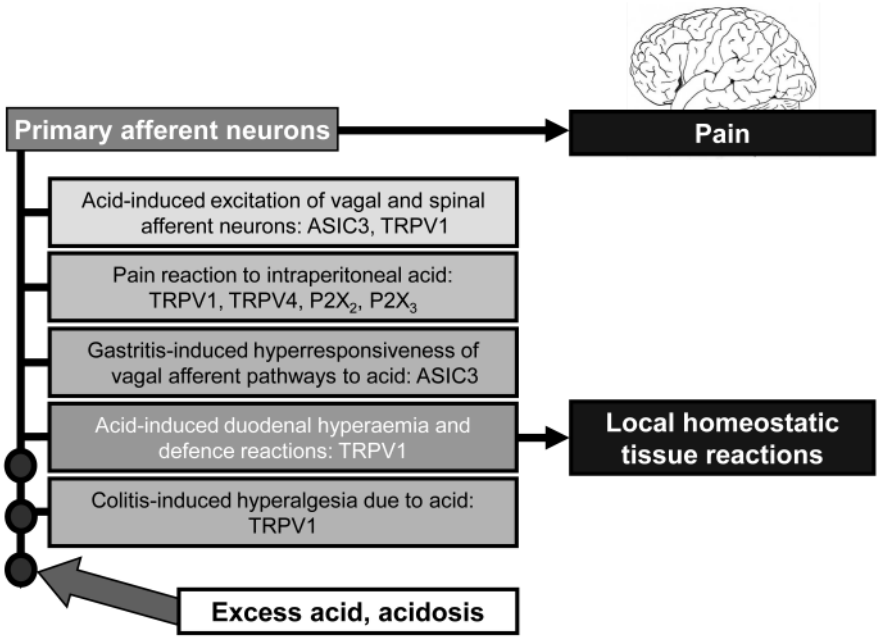

Figure 3.
Schematic overview of the pathophysiological implications of primary afferent neurons and their molecular acid sensors in gastrointestinal function.

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