Introduction

Most tissues would rapidly disintegrate if exposed to the concentrations of acid that are present in the gastric lumen, yet gastric acid is essential for the digestive breakdown of food and elimination of ingested pathogens. The autoggressive potential of gastric acid is kept in check by an elaborate network of mucosal protection mechanisms and the compartmentalization of the oesophago-gastro-duodenal region. Both mechanisms of acid defence require a fast-acting surveillance system, in which acid-sensitive afferent neurons play a particular role [1]. In addition, acid sensors are relevant to the control of gastric acid secretion and to the pathophysiological consequences of tissue acidosis in ischaemia, inflammation and, probably, gastrointestinal (GI) stasis. Besides causing acute pain, acid can sensitize afferent nerve fibres as is the case in inflammatory hyperalgesia [2]. Acid also lowers the threshold of mechanoreceptors in the stomach [3] and contributes to the pain associated with non-cardiac chest pain, gastro-oesophageal reflux, dyspepsia and peptic ulcer.

Acid-sensitive ion channels

Acid-sensitive ion channels (ASICs) are members of the voltage-insensitive, amiloride-sensitive epithelial Na⁺ channel/degenerin family of cation channels [7,8**]. The proton-gated members of this family (Table 1) are encoded by three different genes: ASIC1 (previously termed ASIC), ASIC2 (or BNC1 for brain Na⁺ channel 1) and ASIC3 (or DRASIC for dorsal root-specific ASIC). ASIC1 and ASIC2 each have alternative splice variants denominated as ASIC1a, ASIC1b, ASIC2a (or MDEG1 for mammalian degenerin 1) and ASIC2b (or MDEG2). ASICs consist of two transmembrane (TM) domains (i.e. TM1 and TM2) and a large extracellular loop (Figure 1); their pH sensitivity resides predominantly with Gly430 in the extracellular pre-TM2 region but also with Phe20 and Thr25 in the intracellular pre-TM1 region [7]. Under physiological pH, ASIC3 is blocked by Ca²⁺ bound to the extracellular pore region; protons open ASIC3 by relief of this Ca²⁺ blockade [9*]. Interestingly, lactate generated in ischaemia sensitizes ASICs to acid by decreasing the extracellular Ca²⁺ concentration [10].

Functional channels comprise different ASIC subunits, most of which are expressed (to different degrees) by primary afferent neurons [7,11,12**,13,14**]. The sub-units form homo- or hetero-multimeric channels, which differ in their pH sensitivity and other pharmacological properties [7,8**,12**,13,15,16]. Activation of ASIC1a and ASIC1b by a modest drop in the external pH below 6.9 gives rise to a rapidly inactivating current that is unlikely to account for sustained nociceptor activation [2,7,12**].
ASIC2b (inactive as a homomultimer) forms functional heteromultimers with other ASIC subunits, particularly ASIC3, which is exclusively expressed by small and large dorsal root ganglion (DRG) cells [7,11,12,16,17,18]. The gating of ASIC2b/ASIC3 heteromultimers generates a biphasic current that displays a fast-inactivating and sustained component and is similar to the proton-gated current in DRG cells [2,7,16].

Little is known about the role of ASICs in sensory and nociceptive transduction in the GI tract. Although the cutaneous sensitivity to acid is reduced by disruption of the ASIC3 gene (but not ASIC2) [17,19], the behavioural pain response to intraperitoneal injection of acetic acid is enhanced in ASIC3 knockout mice [18]. The regulation of ASIC function in inflammation is of considerable pathophysiological interest given that, in inflammatory bowel disease (IBD), the expression of ASIC3, but not ASIC1 and ASIC2, is upregulated in the inflamed mucosa [5]. Similarly, experimental inflammation in the skin enhances ASIC expression in sensory neurons, whereas the antiphlogistic drugs aspirin, diclofenac and flurbiprofen counteract the inflammation-induced upregulation of ASICs and inhibit ASIC currents in afferent neurons [11]. Further analysis has revealed that pro-inflammatory mediators, such as nerve growth factor (NGF) and 5-hydroxytryptamine, stimulate ASIC3 transcription in sensory neurons by a direct interaction with the promoter region of the ASIC3 gene [14]. Inflammation also induces expression of FMRFamide-like peptides, and both neuropeptide FF and FMRFamide are able to potentiate H⁺-gated currents in cultured sensory neurons and heterologously expressed ASIC1 and ASIC3 channels [20,21].
The elucidation of ASIC function in health and disease will depend heavily on the availability of selective ASIC inhibitors, and the discovery of psalmotoxin-1 as a potent and selective ASIC1 blocker has been an important advance [22].

**Transient receptor potential cation channels**

The pH sensitivity of ASICs is complemented by two members of the transient receptor potential (TRP) cation channel family: TRPV1 and TRPV4. Originally termed vanilloid receptor-1 because of its unique sensitivity to the vanilloid capsaicin [23,24,25,26,27], TRPV1 is a non-selective cation channel with high permeability for Ca\(^{2+}\). Structurally, it is typified by three ankyrin repeats in the N-terminus, six TM domains and an extracellular re-entrant pore loop between TM5 and TM6 (Figure 1). Functional channels appear to be built of four TRPV1 subunits [26,27], although the existence of heterotetramers of TRPV1 with other members of the TRPV family is becoming increasingly likely [24,25,26,27].

It is of particular interest that TRPV1 displays the functional properties of a polymodal sensor for noxious stimuli, and thereby might play an important role in setting the gain of nociceptive afferents. Thus, TRPV1 is activated not only by vanilloids, such as capsaicin and resiniferatoxin, but also by H\(^{+}\) ions, noxious heat > 43°C, ethanol and arachidonic-acid-derived lipid mediators [23,24,25,26,27,28]. Whereas protons target an extracellular domain of TRPV1, vanilloid and arachidonic-acid-derived agonists bind to an intracellular site of the channel [24,25,26,27,28]. In this way, bradykinin, ATP and NGF could induce hyperalgesia by lowering the temperature threshold of TRPV1 to a level permissive for channel-gating at normal body temperature [2].

Of all the known TRP channels, TRPV1 and TRPV4 are the only ones that respond to acidosis (Table 1). TRPV1 is gated if the extracellular pH falls below 6, in which case

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<th>Acid-sensitive ion channels on primary afferent neurons.</th>
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<tr>
<td>Ion channel</td>
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<td>ASIC3 (DRASIC)</td>
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<td>TRPV4</td>
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<td>P2X2 (P2X(<em>{2,3}), P2X(</em>{2,3}), P2X(_{2,3}))</td>
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<td>P2X3</td>
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<td>P2X4 (P2X(_{4,5}))</td>
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<td>P2X7</td>
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<td>TALK-1 (KCNK16)</td>
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<td>TASK-3 (KCNK9)</td>
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NG, nodose ganglion; TG, trigeminal ganglion.
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a sustained channel current is generated [29,30,39**]. Importantly, mild acidosis (pH 6–7) sensitizes TRPV1 to other stimuli such as capsaicin and heat, and lowers its temperature threshold such that the channel becomes active at normal body temperature [29]. The ability of protons to sensitize TRPV1 to heat and other stimuli, and to activate TRPV1 per se, is mediated by two different extracellular Glu residues in the region linking TM5 with the pore loop (Figure 1) [30]. TRPV4 is concentration-dependently gated in the pH 4–6 range and, in addition, is activated by citrate but not lactate [39**]. Although the response of TRPV4 to heat is enhanced in hypertonic and reduced in hypotonic solutions [40], any interaction of the acid sensitivity of TRPV4 with the osmolarity of its environment awaits to be examined.

Pharmacological studies have indicated that, in the gut, TRPV1 is associated with extrinsic primary afferent neurons. In the DRG, TRPV1 is predominantly expressed by small neurons that give rise to unmyelinated and thinly melanized fibres [23]. TRPV1 is also present in about 40% of nodose ganglion neurons that innervate the rat stomach, but the expression of TRPV1-like immunoreactivity in nodose ganglia is considerably lower than in the DRG [41**]. Thus, the large majority of TRPV1-positive nerve fibres in the rat stomach seems to comprise axons of extrinsic DRG neurons [41**], although TRPV1-like immunoreactivity has been localized to intrinsic enteric neurons of the guinea-pig, porcine and human gut [6**,42,43]. There is ample evidence that capsaicin-induced gating of TRPV1 stimulates extrinsic afferents in the gut [1] and causes abdominal pain in humans [44**,45]. TRPV1-expressing spinal afferents respond to experimentally induced acid backdiffusion in the rat stomach and duodenum, and lead to a prompt increase in mucosal blood flow [1]. TRPV1 appears to be involved in the acid-evoked duodenal hyperaemia, as it is attenuated by the TRPV1 blocker capsazepine [46]; the gastric hyperaemia is left unaltered [47]. It is not yet known whether TRPV1 on spinal afferents participates in the feedback regulation of gastric acid secretion [1] or the acid-induced excitation of vagal afferents and enteric neurons [48,49*]. This is also the case for acid-induced inhibition of gastric emptying, which is mediated by neural reflexes [50].

A role for TRPV1 in pain sensitization can be deduced from the failure of TRPV1 knockout mice to develop thermal hyperalgesia in response to cutaneous inflammation [51,52]. Although paradigms of acid-related GI hypersensitivity have not yet been explored in TRPV1-deficient animals, mice lacking TRPV4 are hyporesponsive to intraperitoneal injection of acetic acid [39**]. IBD is accompanied by an increase in TRPV1-like immunoreactivity on nerve fibres in the submucosa of the colon [4], whereas rectal hypersensitivity and faecal urgency are associated with a rise in both the number of TRPV1-positive nerve fibres in the muscularis and mucosa of the rectum and the proportion of TRPV1-positive enteric neurons [6**]. Inflammation-induced alterations in TRPV1 expression might be mediated by neurotrophic factors that augment the expression of TRPV1 mRNA in cultured sensory neurons and enhance their capsaicin sensitivity [53]; however, in vivo NGF acts largely by a post-transcriptional mechanism involving the mitogen-activated protein kinase p38 [54**]. There is experimental evidence that TRPV1 is involved in rat ileitis caused by Clostridium difficile toxin A [55], rat colitis elicited by dextran sulphate [56] and mouse pancreatitis evoked by caerulein [57].

A therapeutic potential of currently-sought-for TRPV1 channel blockers [58*] can be envisaged from the report that daily intragastric administration of capsaicin (1.75 mg) for five weeks significantly reduced epigastric pain and other symptoms of functional dyspepsia [44**]. This beneficial effect could be explained by downregulation of TRPV1, desensitization of TRPV1 or functional impairment of TRPV1-expressing nociceptive afferent neurons.

**Iontropic purinoceptor ion channels**

P2X purinoceptors are ligand-gated membrane cation channels that open when extracellular ATP is bound. They are assembled as homo-/hetero- trimers or hexamers of various subunits, seven of which (P2X1–P2X7) have been identified at the gene and protein level [59,60**]. Structurally, all P2X subunits are characterized by a long extracellular polypeptide loop between two TM domains (Figure 1). The P2X receptors on nodose ganglion neurons comprise predominantly homomultimeric P2X2 receptors and some heteromultimeric P2X2/3 receptors, whereas homomultimeric P2X3 receptors prevail on DRG neurons [59,61]. In addition to P2X2 and P2X3 receptors, the DRG and nodose ganglia of the rat also express low levels of P2X1, P2X4, P2X5, P2X6 and P2X7 receptors, whereas P2X1, P2X4 and P2X7 receptors appear to be absent from sensory ganglia of the mouse [59,61,62]. The time-course and kinetics of the ATP-gated channel currents differ fundamentally between homo- and heteromultimeric P2X receptors which, in view of the differential P2X subunit distribution in spinal and vagal sensory neurons, explains why ATP-evoked inward currents in nodose ganglion neurons are persistent but those in DRG neurons exhibit transient, persistent or biphase components [59,61].

The activity of most P2X subunits is modulated by alterations in extracellular pH (Table 1). Although the potency of ATP at gating homomultimeric P2X1, P2X3, P2X4, P2X5 and P2X7 receptors is reduced by mild acidification, homomultimeric P2X3 receptors are sensitized to ATP [59,60**,63,64]. Mutational analysis has shown that His319 is particularly important for the ability
of protons to potentiate the agonist effect of ATP at the P2X<sub>3</sub> receptor (Figure 1) [65**]. Because only P2X<sub>2</sub>–4 homomultimers and heteromultimers including P2X<sub>2</sub> (P2X<sub>1,2</sub>, P2X<sub>2/3</sub> and P2X<sub>2,6</sub>) are sensitized by acid, it is primarily P2X<sub>3</sub>-containing purinoceptors that can function as indirect acid sensors in the presence of ATP. This scenario might be of pathophysiological significance because, firstly, ATP is liberated from several cellular sources in response to both physiological and pathological stimuli and, secondly, P2X receptors are upregulated in inflammation. Thus, experimental inflammation in the skin increases the expression of P2X<sub>2</sub> and P2X<sub>3</sub> receptors in DRG cells and augments ATP-evoked currents in these neurons [66].

It has not yet been ascertained whether P2X receptors play a role in GI pain, although ATP can excite vagal and mesenteric afferents [67,68] and IBD is associated with an increase in the number of P2X<sub>3</sub> receptors and P2X<sub>2</sub>/P2X<sub>3</sub> positive nerve fibres and myenteric neurons in the colon [69]. In the inflamed ferret oesophagus, ATP has been found to sensitize vagal afferents to mechanical stimuli [67], although a possible role for acid in sensitizing P2X receptors on GI afferents has not yet been addressed. Importantly, trinitrophenyl-ATP (a P2X<sub>2</sub>/3 and P2X<sub>2,3</sub> receptor blocker) and A-31749 (a non-nucleotide P2X<sub>3</sub> and P2X<sub>2,3</sub> receptor blocker) are able to suppress the nociceptive behaviour provoked by interperitoneal injection of acetic acid in mice, whereas the P2X<sub>1</sub> channel blocker diinosine pentaphosphate is ineffective [70*,71*]. From these effects of TNP-ATP and A-31749, it would appear that the pro-algesic influence of acidic irritants in the peritoneum is mediated by homomultimeric P2X<sub>3</sub> and/or heteromultimeric P2X<sub>2,3</sub> receptors. Therefore, antagonists of these receptors might have therapeutic potential in the treatment of acid-related, inflammation- and ischaemia-induced disturbances of gut function and sensation.

**Acid-sensitive two-pore domain potassium channels**

Two-pore (or tandem-pore) domain potassium (KCNK) channels possess four TM segments (TM1–TM4), two pore-forming loops between TM1 and TM2 as well as between TM3 and TM4, and a large extracellular linker region between TM1 and the first pore-forming loop (Figure 1) [72,73**]. Acid modulates the activity of several KCNK channel family members (Table 1), including TWIK (tandem of pore domains in weak inward rectifier K<sup>+</sup> channel), TREK (TWIK-related K<sup>+</sup> channel), TASK (TWIK-related acid-sensitive K<sup>+</sup> channel), TALK (TWIK-related alkaline pH-activated K<sup>+</sup> channel) and TRAAK (TWIK-related arachidonic acid-stimulated K<sup>+</sup> channel). All KCNK channels appear to exist as dimers, primarily homodimers, although the formation of functional heterodimers (such as TASK-1/TASK-3) has also been reported [74]. Many KCNK channels are thought to be background channels that are independent of membrane voltage, constitutively active and non-inactivating. The resulting ‘leak’ currents play a role in setting the resting membrane potential, as well as the membrane input resistance and, consequently, the excitability of neurons [72,73**].

Importantly, TASK channels are extremely sensitive to variations in extracellular pH in a narrow physiological range. The channels are blocked by very small increases in the extracellular concentration of protons which, in TASK-3, target His98 (Figure 1) adjacent to the first pore-forming loop [72,73**,75–77]. Although TASK inhibition will not result in nerve traffic per se, it is likely to facilitate nerve activity evoked by other stimuli and hence indirectly encode the presence of acid. Other KCNK channel members respond to intracellular acidosis, which inhibits TWIK-1 and TWIK-2 but stimulates TREK-1 and TREK-2 [73**,78**]. Molecular analysis has revealed that lowering of the intracellular pH leads to protonation of Glu306 in the C-terminus of TREK1 (Figure 1), a process that also affects the mechanical and lipid sensitivity of the channel [78**]. In contrast, TRAAK is activated by intracellular alkalization [79], whereas TALK-1, TALK-2 and their splice variants are stimulated by extracellular alkalosis [80–82]. Although any functional implication of KCNK channels in the neural acid surveillance of the GI tract awaits to be proven, various levels of TASK-1, TASK-2, TASK-3, TWIK-1, TWIK-2, TREK-1 and TRAAK mRNA and protein are expressed in the DRGs and gut of humans and rats [79,83,84,85*,86].

**Conclusions**

There is a multitude of neural acid sensors that survey a wide pH range from acidic to alkaline environments. Although ASICs and acid-sensitive TRP channels are directly gated by deviations from physiological pH in the extracellular space, pH-dependent alterations in P2X purinoceptor and KCNK channel (Table 1) activity modulate cell excitability, sensitivity and function. Nearly all of these acid sensors occur in primary sensory neurons supplying the gut, and there is good reason to hypothesize that they are relevant to GI function in health and disease. There is already evidence that, in GI inflammation and hypersensitivity, TRPV1, ASIC3 and P2X<sub>3</sub> are upregulated, but it is largely unknown whether these alterations contribute to the disease process. If a pathophysiological implication can be proved, blockers of the relevant acid-sensitive ion channels might be beneficial in acid-related diseases, as well as in the pain and functional disturbances associated with GI inflammation, ischaemia and stasis.

**Acknowledgements**

Work performed in the author’s laboratory was supported by the Austrian Research Foundation (FWF Grant 14295) and the Jubilee Foundation of the Austrian National Bank (Grant 9858). Evelin Painsipp’s artistry in drawing the figure is greatly appreciated.
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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest


7. In patients with rectal hypersensitivity and faecal urgency, TRPV1 is upregulated in extrinsic afferent nerve fibres and intrinsic enteric neurons of the rectum.


10. A comprehensive review of the molecular physiology and pharmacology of ASICs and their functional implications.


12. Under physiological conditions, ASIC3 is blocked by Ca<sup>2+</sup> bound to a high-affinity binding site on the extracellular side of the channel pore; protons open ASIC3 by relief of this Ca<sup>2+</sup> blockade.


16. A comprehensive investigation of the cellular expression of various ASIC subunits in DRG neurons of the rat.


19. Pro-inflammatory mediators, such as NGF, 5-hydroxytryptamine, interleukin-1 and bradykinin, can promote the transcription of the ASIC3 gene in sensory neurons; 5-hydroxytryptamine and NGF interact directly with the promoter region of the ASIC3 gene.


30. A comprehensive review of the molecular physiology and pharmacology of TRP ion channels, particularly TRPV1, and their functional implications.


32. A review of the molecular physiology and pharmacology of the TRP ion channel TRPV1, with special emphasis on arachidonic acid-derived mediators as endogenous channel activators.


39. N-arachidonoyl-dopamine is an endogenous ligand and activator of the TRP ion channel TRPV1.

The burning sensation of ethanol can, at least in part, be explained by its gastrointestinal TRP ion channel TRPV1 bind to an intracellular site of the channel. Unlike protons, vanilloid and arachidonic acid-derived agonists of the TRP ion channel TRPV1 bind to an intracellular site of the channel.


Unlike protons, vanilloid and arachidonic acid-derived agonists of the TRP ion channel TRPV1 bind to an intracellular site of the channel.


Activation of the cAMP/protein kinase A signalling cascade causes phosphorylation of the TRP ion channel TRPV1 at Ser116 and thus prevents its rapid desensitization.


The protein kinase C signalling pathway is important for regulating the sensitivity of the TRP ion channel TRPV1 to extracellular acid.

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