

Acid-sensitive ion channels in gastrointestinal function

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Deviations from the physiological values of extracellular pH are monitored by multiple acid sensors. Acid-sensing ion channels are activated by moderate acidification, whereas transient receptor potential cation channels, notably TRPV1, are gated by severe acidosis. In contrast, ionotropic purinoceptor (P2X) ion channels, particularly P2X₂, and two-pore domain background K⁺ channels, such as TASK, do not directly signal acidification but rather modulate cell membrane excitability in response to acidosis. These acid sensors, which are expressed by afferent neurons, are most relevant to the regulation of acid secretion, foregut motility and mucosal protection, as well as to gastrointestinal disturbances associated with inflammation, ischaemia and stasis.

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Current Opinion in Pharmacology 2003, 3:618–625

This review comes from a themed issue on
Gastrointestinal pharmacology
Edited by David Grundy and Wendy Winchester

1471-4892/\$ – see front matter
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DOI 10.1016/j.coph.2003.06.008

Abbreviations

ASIC	acid-sensing ion channel
DRG	dorsal root ganglion
GI	gastrointestinal
IBD	inflammatory bowel disease
KCNK	two-pore domain potassium (channel)
NGF	nerve growth factor
TM	transmembrane
TRP	transient receptor potential

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 autoaggressive 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, inflam-

mation 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.

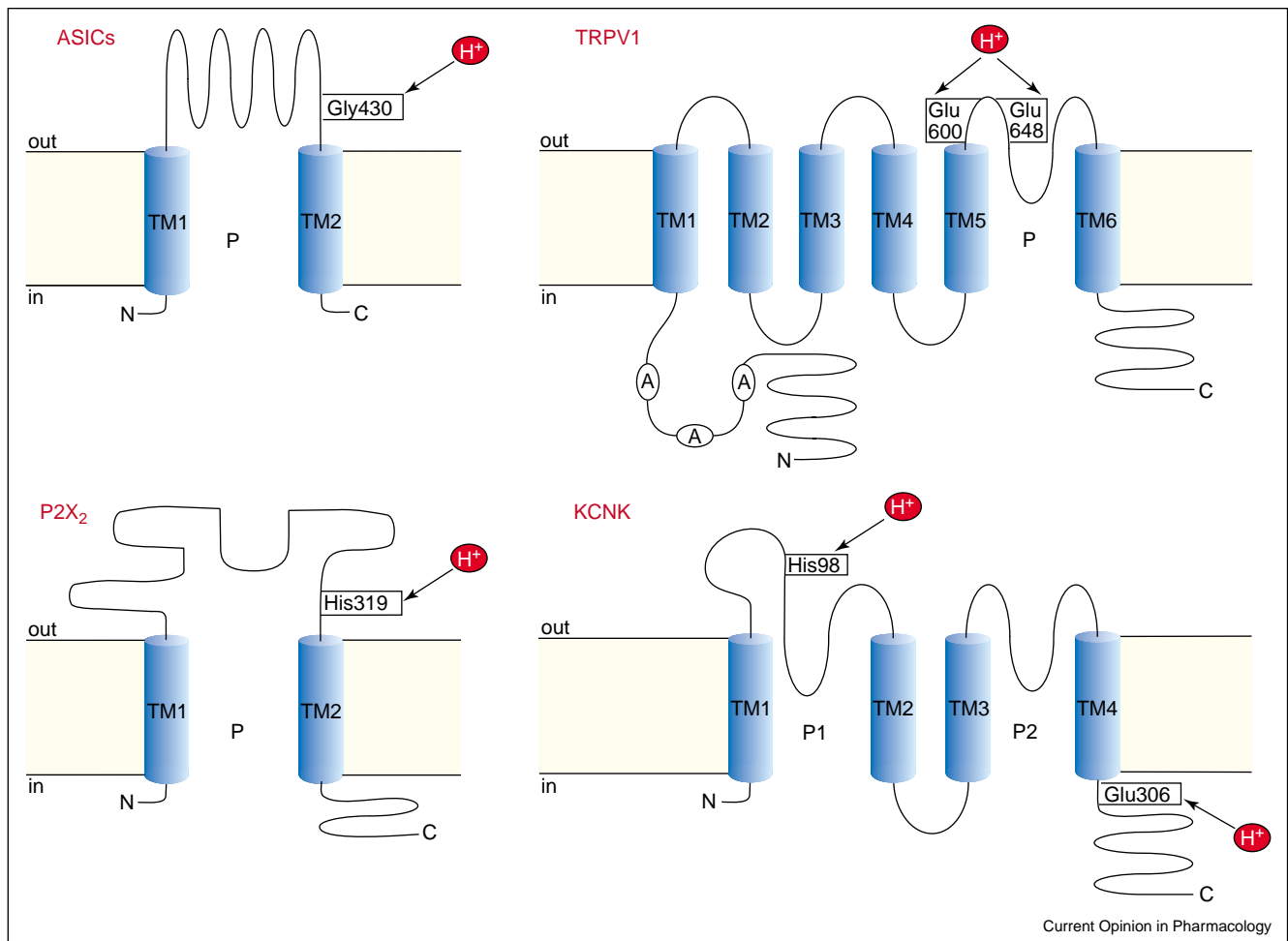
It is known that sensory neurons respond to acidification of their environment, and analysis of acid-induced currents has provided an early hint of the existence of specific proton receptors [2]. In recent years, phenomenal progress has been made in identifying molecular sensors of acidosis (Figure 1; Table 1). Some of these acid-sensitive ion channels are upregulated in GI inflammation and hyperalgesia [4,5,6**], and it appears that neural acid sensors are important targets for novel therapies of chronic abdominal pain. In this review, I highlight some of the most important advances that have been made in this field during the past two years.

Acid-sensing ion channels

Acid-sensing 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 subunits 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**].

Figure 1



Membrane topology of the four major classes of acid-sensitive ion channel subunits. Critical amino acids for proton sensitivity are also indicated. In TRPV1, Glu600 is critical for the effect of mild extracellular acidification (pH 6–7) to sensitize the channel to other stimuli, whereas Glu648 is essential for the opening of the channel at extracellular pH < 6. In TASK-3 (belonging to the KCNK channels), His98 is highly relevant for the ability of extracellular acidification to inhibit the channel, whereas Glu306 determines channel activation by intracellular acidosis in TREK-1, another KCNK channel member. A, ankyrin; P, pore.

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].

Table 1**Acid-sensitive ion channels on primary afferent neurons.**

Ion channel	H ⁺ sensitivity range	Effect of acidosis	Localization to afferent neurons
ASIC1a	Extracellular pH 6–7	Activation	DRG neurons
ASIC1b	Extracellular pH 6–7	Activation	DRG neurons
ASIC2a	Extracellular pH 6–7	Activation	DRG neurons
ASIC2b	Extracellular pH 6–7	Activation	DRG neurons
ASIC3 (DRASIC)	Extracellular pH 6–7	Activation	DRG neurons
TRPV1 (VR1)	Extracellular pH 6–7	Sensitization to other stimuli	DRG neurons
	Extracellular pH < 6	Activation	NG neurons
			TG neurons
TRPV4	Extracellular pH < 6	Activation	DRG neurons
			TG neurons
P2X ₁ (P2X _{1/5})	Extracellular pH < 7	Reduction of ATP potency	DRG neurons
			NG neurons
P2X ₂ (P2X _{1/2} , P2X _{2/3} , P2X _{2/6})	Extracellular pH < 7	Sensitization to ATP	DRG neurons
			NG neurons
P2X ₃	Extracellular pH < 7	Reduction of ATP potency	DRG neurons
			NG neurons
P2X ₄ (P2X _{4/6})	Extracellular pH < 7	Reduction of ATP potency	DRG neurons
			NG neurons
P2X ₅	Extracellular pH < 7	Reduction of ATP potency and efficacy	DRG neurons
			NG neurons
P2X ₇	Extracellular pH < 7	Reduction of ATP potency	NG neurons
TALK-1 (KCNK16)	Extracellular pH < 7.4	Inhibition by acidosis	Not known
		Activation by alkalosis	
TALK-2 (KCNK17)	Extracellular pH < 7.4	Inhibition by acidosis	Not known
		Activation by alkalosis	
TASK-1 (KCNK3)	Extracellular pH < 7.4	Inhibition	DRG neurons
TASK-2 (KCNK5)	Extracellular pH < 7.4	Inhibition	DRG neurons
TASK-3 (KCNK9)	Extracellular pH < 7.4	Inhibition	DRG neurons
TRAAK (KCNK4)	Intracellular pH > 7.4	Activation by alkalosis	DRG neurons
TREK-1 (KCNK2)	Intracellular pH < 7.4	Activation	DRG neurons
TREK-2 (KCNK10)	Intracellular pH < 7.4	Activation	DRG neurons
TWIK-1 (KCNK1)	Intracellular pH < 7.4	Inhibition	DRG neurons
TWIK-2 (KCNK6)	Intracellular pH < 7.4	Inhibition	DRG neurons

NG, nodose ganglion; TG, trigeminal ganglion.

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[•]], TRPV1 is a non-selective cation channel with high permeability for Ca²⁺. 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^{••},28].

It is of particular interest that TRPV1 displays the functional properties of a polymodal sensor for noxious sti-

muli, 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[•],29,30,31[•],32[•]]. Whereas protons target an extracellular domain of TRPV1, vanilloid and arachidonic-acid-derived agonists bind to an intracellular site of the channel [24^{••},25[•],33[•]]. In addition, TRPV1 activity is modulated through intracellular signalling cascades operated by other noxious stimuli such as NGF, prostaglandins, bradykinin and ATP. This is because TRPV1 possesses consensus phosphorylation sites for protein kinase-A, -C and -G, and phosphorylation sensitizes the channel to agonists and prevents its rapid desensitization [24^{••},25[•],34,35[•],36^{••},37,38]. 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

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 hypotonic and reduced in hypertonic 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 myelinated 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 hypo-responsive 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 dextrane 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.

Ionotropic 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 P2X_{2/3} receptors and some heteromultimeric P2X_{2/3} 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 biphasic 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 P2X₂ 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 P2X₂ receptor (Figure 1) [65**]. Because only P2X₂ homomultimers and heteromultimers including P2X₂ (P2X_{1/2}, P2X_{2/3} and P2X_{2/6}) are sensitized by acid, it is primarily P2X₂-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 P2X₂ and P2X₃ 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 P2X₃ receptors and P2X₃-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 P2X₂ receptors on GI afferents has not yet been addressed. Importantly, trinitrophenyl-ATP (a P2X₁, P2X₃ and P2X_{2/3} receptor blocker) and A-31749 (a non-nucleotide P2X₃ and P2X_{2/3} receptor blocker) are able to suppress the nociceptive behaviour provoked by interaperitoneal injection of acetic acid in mice, whereas the P2X₁ 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 P2X₃ and/or heteromultimeric P2X_{2/3} 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⁺ channel), TREK (TWIK-related K⁺ channel), TASK (TWIK-related acid-sensitive K⁺ channel), TALK (TWIK-related alkaline pH-activated K⁺ channel) and TRAAK (TWIK-related arachidonic acid-stimulated K⁺ 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, TREK-2 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 P2X₃ 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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