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REVIEW

TRP channels in neurogastroenterology: opportunities for therapeutic intervention

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The members of the superfamily of transient receptor potential (TRP) cation channels are involved in a plethora of cellular functions. During the last decade, a vast amount of evidence is accumulating that attributes an important role to these cation channels in different regulatory aspects of the alimentary tract. In this review we discuss the expression patterns and roles of TRP channels in the regulation of gastrointestinal motility, enteric nervous system signalling and visceral sensation, and provide our perspectives on pharmacological targeting of TRPs as a strategy to treat various gastrointestinal disorders. We found that the current knowledge about the role of some members of the TRP superfamily in neurogastroenterology is rather limited, whereas the function of other TRP channels, especially of those implicated in smooth muscle cell contractility (TRPC4, TRPC6), visceral sensitivity and hypersensitivity (TRPV1, TRPV4, TRPA1), tends to be well established. Compared with expression data, mechanistic information about TRP channels in intestinal pacemaking (TRPC4, TRPC6, TRPM7), enteric nervous system signalling (TRPCs) and enteroendocrine cells (TRPM5) is lacking. It is clear that several different TRP channels play important roles in the cellular apparatus that controls gastrointestinal function. They are involved in the regulation of gastrointestinal motility and absorption, visceral sensation and visceral hypersensitivity. TRP channels can be considered as interesting targets to tackle digestive diseases, motility disorders and visceral pain. At present, TRPV1 antagonists are under development for the treatment of heartburn and visceral hypersensitivity, but interference with other TRP channels is also tempting. However, their role in gastrointestinal pathophysiology first needs to be further elucidated.

Abbreviations

CGRP, calcitonin gene-related peptide; CNS, central nervous system; DRG, dorsal root ganglia; EC, enterochromaffin; EEC, enteroendocrine; EET, 5,6-epoxyeicosatrienoic acids; ENS, enteric nervous system; EPSP, excitatory postsynaptic potential; FD, functional dyspepsia; GABA, γ-aminobutyric acid; GI, gastrointestinal; IBD, inflammatory bowel disease; IBS, irritable bowel syndrome; ICC, interstitial cells of Cajal; IPAN, intrinsic primary afferent neuron; mGluR1, metabotropic glutamate receptor 1; MP, myenteric plexus; NG, nodose ganglion; PAR, protease-activated receptor; PLC, phospholipase C; SERCA, sarco/endoplasmic reticulum Ca^{2+}-ATPase; SMP, submucosal plexus; TNBS, trinitrobenzene sulfonic acid; TRP, transient receptor potential; TRPA, Ankyrin TRP; TRPC, Canonical TRP; TRPM, Melastatin TRP; TRPML, Mucolipin TRP; TRPP, Polycystin TRP; TRPV, Vanilloid TRP

Introduction

The gastrointestinal (GI) tract is the organ system that controls ingestion and digestion of food, absorption of nutrients, defense against pathogens and removal of indigestible remnants and waste products. Proper regulation of intestinal secretion, absorption, blood flow and motility requires a correct interplay of different humoral and cellular mechanisms including both intrinsic and extrinsic neuronal pathways.

The enteric nervous system (ENS) consists of an extensive neural network embedded in the wall of the gut and controls GI functioning to a large extent independently of the central nervous system (CNS). The ENS consists of different types of neurons and glia, generally organized in two nerve plexus (the myenteric plexus, MP and the submucosal plexus, SMP) with interconnected ganglia that extend along the length of the bowel (Furness, 2006). Interstitial cells of Cajal (ICC), situated at these plexus serve as pacemaker cells by producing rapidly rising, large potential changes that decrementally conduct into the intestinal smooth muscle syncitium (Kito *et al.*, 2005), and are thereby responsible for the generation of intestinal slow waves (Ordog *et al.*, 1999). Another type of ICC within the smooth muscle make close contact with nerve varicosities that innervate these muscle layers, and function as mediators of neuronal input (Faussone-Pellegrini *et al.*, 1989; Burns *et al.*, 1996; Ward *et al.*, 2000). The output of the 'hard-wired' polysynaptic ENS circuits is reflected in the appropriate control of ICC activity, smooth muscle cell contraction or relaxation and secretion.

As a first and crucial step in the intrinsic control of GI function, the intestinal lumen is monitored by different types of enteroendocrine (EEC) cells, including enterochromaffin (EC) cells, scattered throughout the intestinal epithelium. Upon mechanical or chemical stimulation, EC cells release serotonin that activates mucosal processes of intrinsic primary afferent neurons (IPANs), which convey the signal to other neurons in the ENS (Gershon and Tack, 2007). However, this classic model of an intestinal 'reflex arc' in which IPANs serve as 'sensory' neurons has been challenged (Blackshaw *et al.*, 2007). There is growing support for alternative sensory input to the network as different types of enteric neurons are also responsive to mechanical stimuli (Smith *et al.*, 2007; Schemann and Mazzuoli, 2010). Wood (2008) suggested an alternative model by proposing that central pattern generators within the ENS activate the 'hard-wired' basic circuit in recurrent fashion independently of sensory input. As a result of millions of years of evolution and adaptive changes, GI motility is probably controlled by more than one mechanism. The ENS, the ICC network and the smooth muscle cells can therefore be considered as complementary and cooperating control systems that dominate each other depending on specific stimuli (Huizinga and Lammers, 2009). Modulation of either one of these complementary control systems trough intrinsic and extrinsic mediators or pathophysiological conditions will have a major impact on the net output of these integrating systems.

Apart from the intrinsic control of GI functions, there is also an important afferent nerve route, referred to as 'the gut-brain axis', that conveys sensory information to the CNS. Visceral afferent nerves follow two main pathways to the CNS. Vagal afferents have their cell bodies in the nodose ganglia (NG) and jugular ganglia and project centrally to the nucleus tractus solitarius. Spinal afferents, subdivided in splanchnic and pelvic afferents, generally follow the path of intestinal sympathetic and parasympathetic neurons and enter the CNS via the spinal cord. They have their cell bodies in the thoracolumbar and lumbosacral dorsal root ganglia (DRG) respectively. The sensory information conveyed by these visceral afferents is important for the central integration of gut sensation but also helps to coordinate the intrinsic control of gut reflex activity (Blackshaw *et al.*, 2007). However, in diseased states such as inflammation, this information can be dramatically altered, possibly leading to visceral hypersensitivity and pain (Anand *et al.*, 2007; Knowles and Aziz, 2009).

The mammalian transient receptor potential (TRP) superfamily comprises 28 TRP cation channels that can be subdivided into six main subfamilies: the TRPC (Canonical), TRPV (Vanilloid), TRPM (Melastatin), TRPA (Ankyrin), TRPML (Mucolipin) and the TRPP (Polycystin) channels (Ramsey *et al.*, 2006). All TRPs are intrinsic membrane proteins composed of six putative transmembrane segments (S1–S6), cytosolic amino (N) and carboxy (C) termini with a variety of domains (e.g. ankyrin repeats, coiled coiled domains, calmodulin binding sites, etc.) (Owsianik *et al.*, 2006). A putative cation-permeable pore region is located between transmembrane segments S5 and S6. Functional TRP channels form tetramers that are assembled from identical or similar TRP subunits. With exception of TRPM4 and TRPM5, all TRP channels are Ca^{2+}-permeable cation channels. TRP channels can control cell functions by directly permitting Ca^{2+} influx into the cell in response to specific stimuli, or through depolarization of the membrane potential due to cation influx, and influence Ca^{2+} entry through other ion channels, such as voltage-gated Ca^{2+} channels (Nilius and

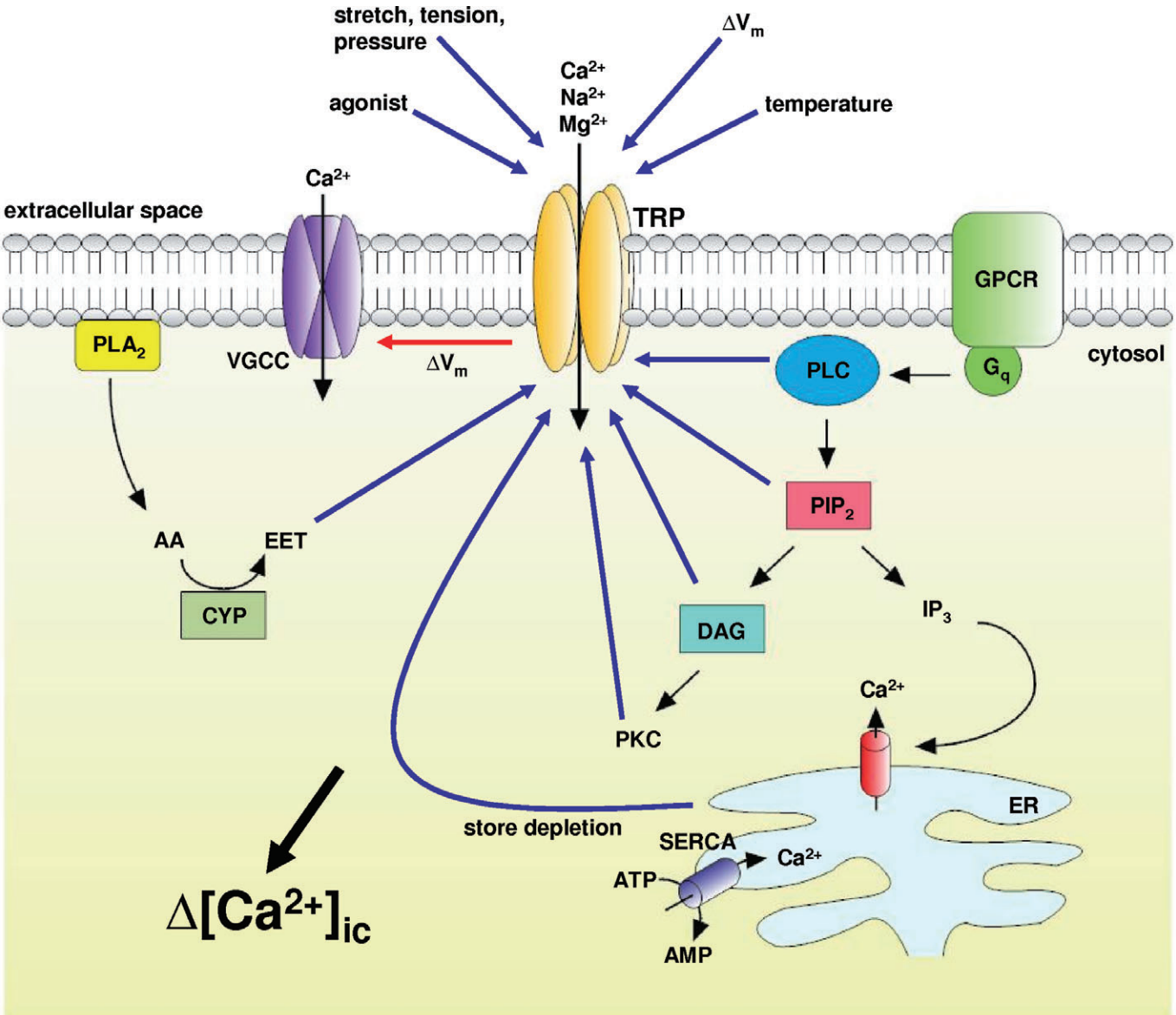

Figure 1

Schematic of the general activation mechanisms of the transient receptor potential (TRP) channels discussed in this review. TRPs are intrinsic membrane proteins that allow the passage of cations. Except for TRPM4 and TRPM5, all TRP channels are Ca^{2+}-permeable cation channels but their selectivity for mono- and divalent cations varies greatly among different TRPs. The activation mechanism of TRP channels is unclear in many cases, but known activators include specific agonists such as capsaicin (TRPV1) and mustard oil (TRPA1), an increase in intracellular Ca^{2+} (TRPM4, 5), temperature (heat: TRPV1, 2, 3, 4, TRPM4, 5; cold: TRPM8, TRPA1), mechanical or osmotic stress (TRPV4, TRPCs?) and phospholipase C (PLC) activation. Cell swelling activates TRPV4 via the PLA_2-pathway. G-protein-coupled receptor (GPCR) activation activates several TRP channels via PLC and diacylglycerol (DAG) dependent mechanisms. Intracellular Ca^{2+} also activates many TRP channels including TRPM5. TRPC3 and TRPC6 are activated by store depletion via a so far unknown mechanism. Several TRP channels are furthermore modulated by depolarization, temperature, intracellular phosphatidylinositol phosphates, such as $PI(4,5)P_2$, but also by inflammatory mediators and steroids. TRP channels control cell functions by directly permitting Ca^{2+} influx into the cell in response to specific stimuli, or through depolarization of the membrane potential due to cation influx. They can also influence Ca^{2+} entry through other ion channels, such as voltage-gated Ca^{2+} channels (VGCC). EET: 5,6-epoxyeicosatrienoic acids; SERCA: sarco/endoplasmic reticulum Ca^{2+}-ATPase. For more details, see text.

Voets, 2005). In many cases, the activation mechanism of TRP channels is unclear (Figure 1), but known activators include specific agonists such as mustard oil (TRPA1) and capsaicin (TRPV1), an increase in intracellular Ca^{2+} (TRPM4, 5), temperature (heat: TRPV1, 2, 3, 4, TRPM4, 5; cold: TRPM8, TRPA1), mechanical or osmotic stress (TRPV4, TRPCs?) and phospholipase C (PLC) activation. TRP channel activity can be further modulated by intracellular phosphatidylinositol phosphates, such as

$PI(4,5)P_2$ and membrane potential, but also by inflammatory mediators, cannabinoids and steroids (Nilius, 2007; Rohacs, 2007; Nilius and Voets, 2008).

Given the nature and action of the TRP channel family, these channels could well be involved in determining the final output of the intrinsic control machinery (ENS, ICC network and smooth muscle) by inducing subtle membrane potential changes. Accumulating evidence furthermore indicates that several of these TRP channels may be highly interesting targets to tackle GI diseases such as visceral hypersensitivity (as reviewed by Blackshaw *et al.*, 2010).

In this review we discuss all mammalian subtypes but focus on those TRP channels, for which there is published evidence for their expression (functionally or the protein/mRNA level) and role in the GI tract. Specifically, we will discuss their function in the control of GI motility, ENS signalling and visceral sensitivity and provide our perspectives on pharmacological targeting of TRPs as a strategy to tackle GI diseases.

TRPC channels

The mammalian TRPC (canonical) subfamily shares most sequence homology with the first TRP channel cloned in *drosophila* (Montell and Rubin, 1989). TRPC channels function as receptor-operated non-selective Ca^{2+}-permeable cation channels that cause membrane depolarization and entry of Ca^{2+} (Birnbaumer, 2009). TRPCs form both homomeric and heteromeric complexes (Strubing *et al.*, 2001; 2003; Goel *et al.*, 2002; Hofmann *et al.*, 2002), which together with the likelihood that TRPCs also functionally overlap, made it difficult to assign specific functions to each of the channel subclasses. In general TRPC channels are activated via different isoforms of PLCs (Venkatachalam *et al.*, 2002). TRPC1, TRPC4 and TRPC5 are activated by receptor-induced PLCs, but are in contrast to TRPC3, TRPC6 and TRPC7, completely unresponsive to diacylglycerol (Hofmann *et al.*, 1999; Venkatachalam *et al.*, 2003; Otsuguro *et al.*, 2008). Several members of the TRPC subfamily can be found in the GI tract, but their role in the regulation of motility is only starting to be unravelled.

Immediately following the discovery of mammalian homologues of the *drosophila* TRP, TRPCs were postulated to be the ion channels responsible for the non-selective cationic conductance that had been recorded in many tissues. The pacemaker current displayed by the slow wave generating ICC in the GI tract was long believed to be initiated by such a voltage-independent, Ca^{2+}-inhibited, non-selective cationic conductance. TRPC4, which was shown to be present in ICC together with TRPC6, was suggested to be a candidate for this current (Torihashi *et al.*, 2002; Walker *et al.*, 2002). Similar to the native pacemaker conductance, TRPC4 expressed in HEK-293 cells was negatively regulated by Ca^{2+}, activated by calmodulin inhibitors and had a similar single channel conductance (Walker *et al.*, 2002). However, another group demonstrated that TRPC4 is activated by PLC and not by Ca^{2+} store depletion or decreases in intracellular Ca^{2+} (Lee *et al.*, 2003a). Also the electrophysiological properties of TRPC4 are subject of debate. Freichel showed an inwardly rectifying current–voltage relationship for TRPC4 whereas Walker *et al.* described outwardly rectifying properties (Freichel, 2001; Walker *et al.*, 2002). Furthermore, because it has been shown that TRPC4 knock-out mice display normal slow waves, TRPC4 appears not to be essential for pacemaker activity (Lee *et al.*, 2005). The role of TRPC4 in ICC is still a matter of debate as evidence is accumulating that a Cl^- current (via Ano1) may be responsible for slow wave initiation (Hwang *et al.*, 2009; Zhu *et al.*, 2009). TRPC4 was also suggested to be implicated in the neurogenic cholinergic control of GI smooth muscle cell contractility (Lee *et al.*, 2005). This was further supported by Tsvilovskyy and colleagues who found expression of TRPC4 and TRPC6 channels in ileal smooth muscle cells. They show that TRPC4 and TRPC6 are gated by muscarinic receptors and are responsible for the muscarinic receptor-induced non-selective cation current that initially depolarizes intestinal smooth muscle cells leading to voltage-activated Ca^{2+} influx and smooth muscle cell contraction (the contribution of TRPC6 is significant but considerably smaller than that of TRPC4) (Tsvilovskyy *et al.*, 2009).

TRPC4 and TRPC6 were also found in the guinea pig ENS. In the SMP, TRPC4/6 immunoreactivity was exclusively expressed by noncholinergic secretomotor neurons while it was present in a small population of both cholinergic and nitrergic neurons in the MP (Liu *et al.*, 2008). TRPC4 could play a role in the release of γ-aminobutyric acid (GABA) from GABAergic nitrergic motor- and interneurons in the ENS (Williamson *et al.*, 1996) by analogy with its role in dendritic GABA release by thalamic interneurons (Munsch *et al.*, 2003).

TRPC1 immunoreactivity was found in cholinergic and noncholinergic secretomotor neurons in the SMP of the guinea pig ENS (Liu *et al.*, 2008). Interestingly, TRPC1 mediates metabotropic glutamate receptor 1 (mGluR1)-induced slow excitatory postsynaptic currents in Purkinje neurons (Kim *et al.*, 2003). Because mGluR1 is also expressed in submucous neurons of the ENS, it may be worth-

while to investigate whether also here TRPC1 is involved in mGluR1-mediated, non-purinergic slow excitatory postsynaptic potentials displayed by some vasoactive-intestinal peptide positive secretomotor neurons in the guinea pig (Hu *et al.*, 1999; Foong and Bornstein, 2009). In the MP, TRPC1 immunoreactivity is widely distributed and localized to neurons with a cholinergic, calretinin and nitrergic neurochemical phenotype.

Less information is available about the other members of the TRPC subfamily. TRPC2 mRNA was found in longitudinal muscle – myenteric plexus (LMMP) preparations and in isolated myenteric ganglia of guinea pig (Liu *et al.*, 2008). However, TRPC2 is irrelevant in human physiology as its complete gene has been lost from the Old World monkey and human genomes, in which its remnants constitute a pseudogene (Yildirim and Birnbaumer, 2007). While TRPC3 mRNA and protein was also detected in LMMP, the SMP and myenteric ganglia, immunohistochemical analysis showed that TRPC3 immunoreactivity was exclusively expressed by neuropeptide Y immunoreactive neurons in the guinea pig SMP (Liu *et al.*, 2008). TRPC5, which was also proposed as a candidate for muscarinic receptor activation induced non-selective cation current in murine stomach (Lee *et al.*, 2003b), was found in LMMP, SMP and myenteric ganglia using both RT-PCR and Western blotting (Liu *et al.*, 2008). Finally, TRPC7 is suggested to be present on GI smooth muscle (Walker *et al.*, 2001; Liu *et al.*, 2008).

Whether GI TRPC channels will become valid therapeutic targets awaits further clarification of their role in gut physiology and pathophysiology. However, the interference with smooth muscle TRPCs to modify contractility could particularly be interesting to treat various GI motility disorders.

For an overview, see Table 1.

Table 1

TRPC channels in neurogastroenterology

	Proposed location	Proposed function
TRPC1	Enteric neurons of the SMP and MP	mGluR1-induced slow EPSPs
TRPC2	LMMP, MP	?
TRPC3	SMP, MP, NPY neurons	?
TRPC4	ICC, smooth muscle, noncholinergic secretomotor neurons in the SMP, enteric neurons in the MP	Pacemaker current, muscarinic receptor-induced smooth muscle cell depolarization, GABA release
TRPC5	SMP, MP, smooth muscle	Muscarinic receptor-induced smooth muscle cell depolarization
TRPC6	ICC, smooth muscle, SMP, MP	Muscarinic receptor-induced smooth muscle cell depolarization
TRPC7	Smooth muscle	?

EPSP, excitatory postsynaptic potential; GABA, γ-aminobutyric acid; ICC, interstitial cell of Cajal; LMMP, longitudinal muscle-myenteric plexus; mGluR1, metabotropic glutamate receptor 1; MP, myenteric plexus; NPY, neuropeptide Y; SMP, submucosal plexus.

TRPV channels

Six mammalian genes TRPV1–TRPV6 code for the members of the TRPV subfamily. These channels contain six ankyrin repeats in their cytosolic N-termini. The first four (TRPV1–TRPV4) are polymodal thermo- and chemo-sensitive channels that are non-selective for cations and modestly permeable to Ca^{2+}. On the other hand, TRPV5 and TRPV6 are highly Ca^{2+}-selective channels and tightly regulated by intracellular [Ca^{2+}] (Nijenhuis *et al.*, 2005). For detailed reviews about the members of the TRPV subfamily, see following reviews (O'Neil and Heller, 2005; Vennekens *et al.*, 2008; Vriens *et al.*, 2009).

TRPV1, the founding member of the mammalian TRPV channels, is predominantly expressed on unmyelinated and some thinly myelinated sensory neurons that can be activated by capsaicin, noxious heat, acidosis (pH < 5.9), depolarization and endovanilloids (Caterina *et al.*, 1997; Tominaga *et al.*, 1998; Voets *et al.*, 2004). Apart from signalling to the CNS, activation of TRPV1 on these afferents also provokes local efferent-like effects that control peripheral effector mechanisms, GI motility and secretion via the release of tachykinins and calcitonin gene-related peptide (CGRP) (Holzer, 1988; 2006; Bartho *et al.*, 1992; Bartho and Holzer, 1995; de Man *et al.*, 2008).

Several inflammatory mediators and factors associated with hyperalgesia (e.g. bradykinin, 5-HT, neuronal growth factor, tryptase), but also mild acidosis are able to sensitize TRPV1 and enhance the probability of channel gating by heat and capsaicin (Chuang *et al.*, 2001; Ji *et al.*, 2002; Amadesi *et al.*, 2004; 2006; Sugiuar *et al.*, 2004). Because TRPV1 was suggested to play a crucial role in nociception based on its expression on mostly unmyelinated visceral afferents (Ward *et al.*, 2003; Brierley *et al.*, 2005; Christianson *et al.*, 2006), a vast number of studies investigating its role in visceral sensation and pain emerged (Caterina *et al.*, 2000). (Reviews by Di

Marzo *et al.*, 2002; Geppetti and Trevisani, 2004; Holzer, 2008b; Blackshaw *et al.*, 2010).

Animal experiments have demonstrated an important role for TRPV1 both in gastro-esophageal (Bielefeldt and Davis, 2008), small intestinal (Rong *et al.*, 2004) and colonic (Jones *et al.*, 2005) visceral mechanosensitivity as well as in hypersensitivity induced by intracolonic administration of zymosan (Jones *et al.*, 2007) or neonatal irritation of the colon (Winston *et al.*, 2007). In addition, TRPV1 was shown to be up-regulated in nociceptive visceral afferents of rodents with experimental trinitrobenzene sulfonic acid (TNBS)-induced colitis (Miranda *et al.*, 2007; De Schepper *et al.*, 2008a). De Schepper *et al.* showed that acute TNBS-induced colitis increased the response to colorectal distention in rat pelvic afferent C fibres but not in Aδ fibres. This inflammation-induced increase in mechanosensitivity was reduced by the TRPV1 antagonist BCTC (De Schepper *et al.*, 2008b). Intriguingly, and indicative of the controversy in this area of research, a similar *in vivo* study by Sengupta *et al.* did not demonstrate any change in mechanosensitivity after TNBS-induced colitis (Sengupta *et al.*, 1999). In agreement with the latter study, mechanosensitivity of nociceptive serosal and mesenteric colonic splanchnic afferents to graded stimuli was shown to be unaffected during dextran sulfate sodium colitis in rats (Phillis *et al.*, 2009). However, TRPV1 seems to be an important amplifier of noxious stimuli as the antagonist SB-750364 inhibited mechanosensitivity and spontaneous neuronal discharge only during inflammation but not in healthy conditions (Phillis *et al.*, 2009). Taken together, it could well be that the effect of TRPV1 antagonists may only become significant after TRPV1 up-regulation, which is not necessarily associated with obvious inflammation. In rats, stress-induced visceral hypersensitivity that is dependent on mast cell degranulation and subsequent TRPV1 activation also occurs in the absence of overt inflammation (van den Wijngaard *et al.*, 2009). Similarly, up-regulation of TRPV1 in visceral afferents has also been observed without overt inflammation as is typical of patients with irritable bowel syndrome (IBS) (Akbar *et al.*, 2008).

As far as human tissue is concerned, altered TRPV1 expression on nerve fibres has been demonstrated in biopsies from patients with rectal hypersensitivity (Chan *et al.*, 2003) or inflammatory bowel disease (IBD) (Yiangou *et al.*, 2001) and shown to correlate with the degree of abdominal pain in IBS (Akbar *et al.*, 2008). By infusing capsaicin in the human small intestine, enhanced chemonociception in functional dyspepsia (FD) patients compared with controls has been reported (Schmidt *et al.*, 2004; Hammer *et al.*, 2008). It is unclear whether TRPV1 receptors contribute to symptom generation in FD, either indirectly through sensitization of mechanosensitive afferents, or directly, through activation by luminal factors such as gastric acid. Symptomatic benefit of long-term administration of capsaicin tablets was also shown in FD and attributed to desensitization of gastric nociceptive C-type fibres (Bortolotti *et al.*, 2002). This 'desensitization' is most probably caused by defunctionalization or ablation of sensory afferent fibres due to repeated stimulation of TRPV1 (Holzer, 2008a).

TRPV1-expressing afferents also innervate the esophagus. Here, TRPV1 immunoreactivity is increased in mucosal biopsies from patients with erosive (Matthews *et al.*, 2004) and non-erosive esophagitis (Bhat and Bielefeldt, 2006) and it is expected that TRPV1 also plays a key role in the pathogenesis of symptoms associated with reflux (Ang *et al.*, 2008). In keeping with this hypothesis, intra-esophageal capsaicin installation was found to induce symptoms of heartburn and chest pain in healthy volunteers (Kindt *et al.*, 2009). The proposed role of TRPV1 is further supported by a recent study showing that acid-induced activation of TRPV1 receptors in feline esophageal mucosa results in substance P and CGRP release, presumably arising from submucous neurons, together with the release of platelet activating factor from esophageal epithelial cells, which also turned out to express TRPV1 (Cheng *et al.*, 2009). The release of pro-inflammatory mediators via TRPV1 activation probably also plays a role in acid-induced esophagitis because TRPV1 null mice develop less severe esophagitis compared with wild-type mice (Fujino *et al.*, 2006). Based on these observations, TRPV1 antagonists are under development for the treatment of heartburn and visceral hypersensitivity, among other therapeutic targets (Gunthorpe and Szallasi, 2008). Although hyperthermia was reported with several TRPV1 antagonists, there is new evidence that TRPV1 antagonists that are devoid of this adverse effect can be synthesized. It is, however, still too early to make conclusions regarding their efficacy (Khairatkar-Joshi and Szallasi, 2009). Crucial in the current drug development for TRPV1 antagonists that is aimed at dissociating hyperthermia effects from anti-hyperalgesic efficacy, is to differentiate between the physiological role and pathological manifestation of TRPV1. Specific intervention with the latter population is definitely of high interest from a therapeutic point of view (Holzer, 2008a).

Apart from its presence on esophageal epithelial cells, TRPV1 was also found to be expressed in human gastric oxyntic cells and mucous secreting epithelial cells in the rat stomach (Nozawa *et al.*, 2001; Kato *et al.*, 2003; Faussone-Pellegrini *et al.*,

2005). Together with neuronal mechanisms, this particular epithelial location of TRPV1 is suggested to be involved in the regulation of gastric chlorhydropeptic secretion (Raybould and Tache, 1989; Horie *et al.*, 2004; Faussone-Pellegrini *et al.*, 2005).

Taken together, these data all point to an important role for TRPV1 in the modulation of mechanosensitivity, chemosensitivity and pain in pathological states. However, some controversy about the details of TRPV1's involvement in mechanosensation and nociception in different models still exists. Questions as to whether increased TRPV1 function during inflammation is due to higher open probability of the channel leading in turn to increased responses to inflammatory mediators such as 5-HT will need to be determined in future studies (Sugiuar *et al.*, 2004; Coldwell *et al.*, 2007). Also the interaction of TRPV1 with other signalling systems, especially the protease-activated receptor (PAR) has to be addressed in more detail (Amadesi *et al.*, 2004; 2006). Also the relationship between TRPV1 and the cannabinoid system in the GI tract (Sanger, 2007) is of increasing interest as it has been shown that endocannabinoids are produced within the ENS (Boesmans *et al.*, 2009; Hong *et al.*, 2009). Interference with TRPV1 function for pure analgesic purposes furthermore warrants a better understanding of the physiological role of TRPV1 in other than neuronal tissues (Wong and Gavva, 2009).

TRPV4 is another member of the TRPV subfamily that is also implicated in mechanosensation and pain (Alessandri-Haber *et al.*, 2003; Suzuki *et al.*, 2003a). The channel can be activated by endogenously produced 5,6-epoxyeicosatrienoic acid, an arachidonic acid metabolite (Watanabe *et al.*, 2003), as well as by the synthetic phorbol ester 4 α-phorbol 12,13-didecanoate (Watanabe *et al.*, 2002), bisandrographolide A (Smith *et al.*, 2006) and by physical stimuli (Vriens *et al.*, 2004). The channel is expressed in sensory neurons and TRPV4 null mice display decreased cutaneous pain and an increased threshold for somatic mechanical nociception, all of which firmly links TRPV4 to mechanotransduction (Suzuki *et al.*, 2003a,b; Grant *et al.*, 2007).

However, apart from its role in somatic nociception, recent evidence also points towards a role for TRPV4 in visceral nociception (Blackshaw *et al.*, 2010). Brierley *et al.* investigated whether TRPV4-expressing serosal and mesenteric afferents in the colon were visceral nociceptors (Brierley *et al.*, 2008). Consistent with the expression of TRPV4 on visceral nerve endings, they found that mechanosensory responses of these afferents were dramatically reduced in TRPV4-deficient mice, while vagal and pelvic mucosal and muscular afferents showed no deficit. Because the effect of a non-selective TRP channel blocker ruthenium red, known to reduce mechanosensitivity of the presumed visceral nociceptors, was completely lost in TRPV4 null mice, it is conceivable that these neurons exclusively use TRPV4, and no other TRP channels, for their mechanosensation. Cenac *et al.* also showed that TRPV4 activation by 4 α-phorbol 12,13-didecanoate causes visceral allodynia and hyperalgesia and using a siRNA approach, they also demonstrated that TRPV4 is involved in visceral nociception in response to colorectal distention (Cenac *et al.*, 2008). Similar to its expression in mice, TRPV4 immunoreactivity was also found in the human colon, and was suggested to be enriched in resections from IBD patients (Brierley *et al.*, 2008). If expression is indeed increased in Crohn's disease and/or ulcerative colitis, the TRPV4 channel should be further explored as a target for pain relief in these patients.

PAR2 activation, which is implicated in the generation of visceral hypersensitivity by mediators released from colonic biopsies of patients with IBS (Cenac *et al.*, 2007; Buhner *et al.*, 2009), potentiates the response of cultured DRG neurons on TRPV4 agonists (Grant *et al.*, 2007). In agreement, Cenac *et al.* and Sipe *et al.* furthermore showed that PAR2-induced hypersensitivity is mediated by the activation of TRPV4 (Cenac *et al.*, 2008; Sipe *et al.*, 2008). TRPV4 and PAR2 are co-expressed on the majority of spinal afferents innervating the murine colon and TRPV4 deletion or antagonism completely abolishes the sensitizing effects of PAR2 activation in these neurons (Sipe *et al.*, 2008). However, because PAR2-induced Ca^{2+} transients were not inhibited by TRPV4-targeted siRNA in DRG neurons, parallel, yet incremental, pathways for PAR2 and TRPV4 are suggested at the cellular level (Cenac *et al.*, 2008). These observations suggest that TRPV4 can be considered a therapeutic target in GI disorders that involve elevated production or release of proteases, such as IBS (Roka *et al.*, 2008). Apart from neuronal expression, TRPV4 is also expressed by brush-bordered epithelial cells but its function in the mucosa has not been addressed (Cenac *et al.*, 2008). Interestingly, PAR4 was recently shown to counterbalance TRPV4 and PAR2 elicited allodynia and hyperalgesia (Auge *et al.*, 2009). Auge *et al.* demonstrated that PAR4 is present on murine colonic afferents expressing both PAR2 and TRPV4. Intracolonic administration of a subinflammatory dose of a PAR4 agonist reduced the visceromotor response to colorectal distention and inhibited visceral hyperalgesia induced by PAR2 or TRPV4 activation (Auge *et al.*, 2009).

TRPV2 is expressed in TRPV1-negative thinly myelinated neurons of rat DRG, TG and NG (Caterina *et al.*, 1999; Ichikawa and Sugimoto, 2003;

Table 2
TRPV channels in neurogastroenterology

	Proposed location	Proposed function
TRPV1	Visceral afferents, esophageal epithelial cells, oxyntic cells	Visceral chemo-, mechano-and nociception, ?
TRPV2	TRPV1-negative visceral afferents, enteric neurons in the SMP and MP	?
TRPV4	Visceral afferents, epithelial cells	Visceral mechanosensation and nociception, ?
TRPV5, 6	Epithelium	Ca^{2+} absorption

MP, myenteric plexus; SMP, submucosal plexus.

Lewinter *et al*., 2004; Zhang *et al*., 2004) and is activated, at least in rodents, by noxious heat (>52°C). Kashiba *et al*. found TRPV2-positive neurons in the rat MP and SMP, some of which were also immunoreactive for Ca^{2+}-binding protein calbindin (Kashiba *et al*., 2004). However, calbindin is not a conclusive marker for IPANs in the rat ENS, therefore the claim that these neurons are intrinsic sensory neurons remains speculative. Because heat stimuli above 52°C are highly unlikely to occur in the gut, a role in mechanosensation as proposed by Muraki is more likely in the gut (Muraki *et al*., 2003). However, while heat and mechano-activation of TRPV2 remain controversial, the channel is consistently activated by 2-aminoethoxydiphenyl borate and tetra-hydrocannabinol, the psychoactive compound in marijuana (Neeper *et al*., 2007). Future studies will have to provide insight into the role of TRPV2 in the gut.

At present, there is no evidence for expression of TRPV3 in the gut. The last two members of the TRPV family (TRPV5 and TRPV6) are mainly involved in Ca^{2+} absorption across epithelial cells (Suzuki *et al*., 2008). TRPV5 and TRPV6 are located on the brush border membrane of enterocytes and are considered rate-limiting in active intestinal Ca^{2+} absorption (Hoenderop and Bindels, 2008). Mice lacking TRPV6 display a 60% decrease in intestinal Ca^{2+} absorption, lower bone mineral density, deficient weight gain and decreased fertility (Bianco *et al*., 2007). For an overview, see Table 2.

TRPM channels

The TRPM family consists of eight different channels, TRPM1–TRPM8. TRPM channels exhibit highly variable permeability to Ca^{2+} and Mg^{2+}, ranging from Ca^{2+} impermeable (TRPM4, 5) to highly Ca^{2+} and Mg^{2+} permeable (TRPM6, 7). Contrary to TRPCs and TRPVs, TRPMs do not contain ankyrin repeats within their N-terminal domain. The main characteristics of the different members of the TRPM subfamily have been reviewed extensively (Aarts and Tymianski, 2005; Kraft and Harteneck, 2005; McNulty and Fonfria, 2005).

TRPM5 is a highly temperature-sensitive, heat-activated channel expressed in taste cells of the tongue and is involved in the signal transduction of sweet, bitter and umami tastes (Zhang *et al*., 2003; Talavera *et al*., 2005; Damak *et al*., 2006). Downstream of the taste receptor, an increase in intracellular Ca^{2+} leads to TRPM5 activation, which in turn enhances membrane depolarization and signal propagation by synaptic transmission or paracrine peptide release. It is proposed that nutrient sensing in the GI tract resembles taste signalling on the tongue (Rozengurt and Sternini, 2007; Sternini *et al*., 2008). Indeed, together with other taste signalling molecules, TRPM5 is expressed in epithelial cells of the gut (Bezencon *et al*., 2007; Jang *et al*., 2007; Young *et al*., 2009), and similar to their function in lingual taste cells, the GI taste signalling elements also respond to tastants in the gut lumen (Jang *et al*., 2007; Margolskee *et al*., 2007). TRPM5 is shown to be present in human duodenal L cells, where its expression is inversely related to the blood glucose concentration at least in subjects with type 2 diabetes (Jang *et al*., 2007; Young *et al*., 2009). However, important species differences do exist as in the murine gut TRPM5 is not colocalized with any of the classical markers for EEC cells such as chromogranin A, 5-HT, glucagon-like peptide 1, ghrelin and cholecystokinin (Bezencon *et al*., 2007; 2008; Kokrashvili *et al*., 2009). In mouse, TRPM5 is mostly expressed in brush cells (Kaske *et al*., 2007; Bezencon *et al*., 2008), also referred to as tufted or caveolated cells (Sbarbati and Osculati, 2005a,b; Morroni *et al*., 2007). Recently, it has been shown to be essential for the release of endogenous opioids from duodenal epithelial cells that contain enkaphelin and uroguanilyn (Kokrashvili *et al*., 2009).

Apart from TRPC4 and TRPC6 (see above), also TRPM7 was suggested to be involved in the pacemaker current of ICC. Kim *et al*. demonstrated that TRPM7 is expressed in murine ICC and that the cation selectivity of pacemaker currents in primary cultured ICC was similar to that of TRPM7 (Kim *et al*., 2005). Targeting TRPM7 using a RNA interference approach, resulted in inhibition of pacemaking activity. TRPM7 is, however, best described for its role in cell viability and magnesium homeostasis (Nadler *et al*., 2001; Schmitz *et al*., 2003; Jin *et al*., 2008). Therefore, skepticism has arisen regarding

the effect of siRNA experiments on cell viability and subsequent loss of pacemaker current (Farrugia and Kraichely, 2005).

Another member of the TRP family, TRPM8, initially identified in prostate cancer (Tsavaler *et al.*, 2001), is expressed in DRG, TG and in papillae of the tongue and can be activated by cooling and menthol (McKemy *et al.*, 2002; Peier *et al.*, 2002). Mice lacking TRPM8 exhibit deficient behavioural responses to cold temperatures (Bautista *et al.*, 2007; Colburn *et al.*, 2007; Dhaka *et al.*, 2007). Mint leaves have been used for ages because of their therapeutic and soothing effects. The ancient Romans, for example, chewed mint leaves to freshen their breath after a meal but also to aid digestion. Peppermint oil, with menthol as major constituent, reduces gastric spasms and slows small intestinal transit (Goerg and Spilker, 2003; Hiki *et al.*, 2003). Menthol is also present in herbal drugs designed to treat abdominal discomfort and pain (Schemann *et al.*, 2006; von Arnim *et al.*, 2007; Pilichiewicz *et al.*, 2007). The concept of using naturally occurring TRPM8 ligands, such as menthol, to inhibit hypermotility states is attractive. However, the expression and role of TRPM8 in the GI tract and its involvement in the clinical effects of herbal medicines are still controversial. TRPM8 mRNA was detected in the fundus (and colon) of rat and was implicated in cooling-induced contractions of fundic muscle strips (Mustafa and Oriowo, 2005). Zhang *et al.* also found TRPM8 mRNA in murine NG, small intestinal and stomach mucosa but Penuelas *et al.* failed to confirm its expression although the same primer pairs were used (Zhang *et al.*, 2004; Penuelas *et al.*, 2007). The latter group also showed that menthol produces slow but long-lasting relaxations of the mouse colon and consequently concluded this phenomenon to be TRPM8-independent (Penuelas *et al.*, 2007).

The role of TRPM6 in the GI tract is well established. TRPM6 is important for Mg^{2+} absorption across intestinal epithelial cells and hypomagnesaemia with secondary hypocalcemia is one of the best studied channelopathies so far (Quamme, 2008).

Given the high number of mast cells in the gut wall, TRPM4 might sensitize or modulate visceral afferent activity. In mice lacking TRPM4, bone marrow-derived mast cells had augmented degranulation and released more histamine, leukotrienes and tumour necrosis factor due to increased Ca^{2+} entry after FcepsilonRI stimulation (Vennekens *et al.*, 2007). TRPM4 is also involved in the migration of bone marrow-derived mast cells by regulation of Ca^{2+}-dependent actin cytoskeleton rearrangements (Vennekens *et al.*, 2007; Shimizu *et al.*, 2009). TRPM4 could be considered a therapeutic target in GI disorders that involve mast cell activation/degranulation, such as food allergies, mastocytosis and IBS (Bischoff, 2009). For an overview, see Table 3.

Table 3
TRPM channels in neurogastroenterology

	Proposed location	Proposed function
TRPM4	Mast cells	Migration and degranulation of mast cells.
TRPM5	EEC cells, brush cells	Nutrient sensing, release of endogenous opioids.
TRPM6	Epithelium	Mg^{2+} absorption
TRPM7	ICCs	Pacemaker current
TRPM8	Visceral afferents	?

EEC, enteroendocrine; ICC, interstitial cell of Cajal.

TRPA1

The TRPA family contains only one mammalian member, TRPA1. The channel contains 14–16 NH2 terminal ankyrin repeats (Story *et al.*, 2003), an unusual structural feature that might be relevant to its proposed mechano-sensory role (Nagata *et al.*, 2005). TRPA1 is activated by noxious cold (17 ± 1°C) and is present on sensory neurons, primarily in subsets expressing TRPV1 (Story *et al.*, 2003; Bandell *et al.*, 2004; Nagata *et al.*, 2005; Bautista *et al.*, 2006; Karashima *et al.*, 2009). Activation of TRPA1 represents, together with TRPM8 activity, the main mechanism underlying cold sensing (Dhaka *et al.*, 2006; Wrigley *et al.*, 2009). Chemical activators of TRPA1 include isothiocyanates (the pungent compounds in mustard oil, wasabi and horseradish), methyl salicylate (in winter green oil), cinnamaldehyde (in cinnamon), allicin and diallyl disulphide (in garlic), acrolein (an irritant in exhaust fumes and teargas), nicotine, menthol and tetrahydrocannabinol (Bandell *et al.*, 2004; Jordt *et al.*, 2004; Macpherson *et al.*, 2005; Bautista *et al.*, 2006; Karashima *et al.*, 2007; Talavera *et al.*, 2009). For a more detailed review, see (McMahon and Wood, 2006).

TRPA1 was found to be present in vagal, splanchnic and pelvic visceral afferents in mouse (Brierley *et al.*, 2009; Cattaruzza *et al.*, 2009), rat (Kondo *et al.*, 2009) and guinea pig (Yu and Ouyang, 2009; Yu *et al.*, 2009). It is mainly expressed in afferents with mucosal and serosal/mesenteric mechanoreceptive fields (Brierley *et al.*, 2009) and

colocalization with CGRP, substance P, tyrosine kinase A and TRPV1 was described (Brierley *et al.*, 2009; Cattaruzza *et al.*, 2009; Kondo *et al.*, 2009). Recent observations indicate that the mechanosensory function is altered in specific subtypes of visceral afferents of TRPA1 knock-out mice (Brierley *et al.*, 2009). TRPA1 thus contributes to the detection of low- and high-intensity mechanical stimuli depending on the afferent ending in which they are expressed. Especially the involvement of TRPA1 in low-intensity mechanical stimuli (~tactile) is surprising as TRPA1 was believed to be only involved in noxious stimuli (Kwan *et al.*, 2006). The role of TRPA1 in visceral mechanosensation was also explored in the stomach of rats where gastric distention induced ERK1/2 activation in TRPA1 expressing DRG and NG neurons. Interestingly, antisense knock-down of TRPA1 suppressed this activation in DRG, but not in NG neurons (Kondo *et al.*, 2009).

Together with the recent documentation of the mechanosensory function of TRPA1, also its role in visceral hypersensitivity is extensively studied (Blackshaw *et al.*, 2010). TRPA1 agonists induce mechanical hypersensitivity, which is exacerbated in inflammatory conditions, as seen after TNBS treatment (Brierley *et al.*, 2009; Cattaruzza *et al.*, 2009) but is not involved in distention-induced pain in mice under basal conditions (Cattaruzza *et al.*, 2009). The channel is furthermore shown to play a pivotal role in bradykinin-induced mechanical hypersensitivity but does not contribute to the actual bradykinin-induced responses (Brierley *et al.*, 2009; Yu and Ouyang, 2009). Yu *et al*. recently demonstrated that TRPA1 plays an important role in mast cell activation-induced long-lasting mechanical hypersensitivity of vagal nodose C-fibres in the guinea-pig esophagus (Yu *et al.*, 2009). Mast cell tryptase-induced PAR2 activation is proposed as a mechanism for TRPA1 sensitization, which was confirmed by Cattaruzza *et al*. who showed that PAR-induced hyperalgesia was absent in TRPA1 null mice (Cattaruzza *et al.*, 2009). However, PAR2 activation did not change mechanosensory function in mice lacking TRPA1 in the study by Brierley and colleagues. Moreover, the TRPA1-deficient afferents in these mice also showed similar responses to PAR2 activation compared with wild-type afferents (Brierley *et al.*, 2009). Similarly as for TRPV channels, some controversy about the involvement of PAR2 still exists. Whether regional differences (colonic/splanchnic vs. esophageal/vagal), species differences (guinea pig vs. mouse) or different background of TRPA1-deficient mice are the source of these opposing observations has to be subject of further research. If confirmed in humans, the involvement of TRPA1 in mechanosensitivity would make this a target to consider in disorders characterized by enhanced mechanosensitivity, which is the case in many functional GI disorders (Anand *et al.*, 2007).

Apart from visceral afferents, expression of TRPA1 was also found in human and rat EC cells, in which TRPA1 agonists cause Ca^{2+} influx and 5-HT release (Nozawa *et al.*, 2009). It is proposed that TRPA1 agonists delay gastric emptying via such serotonergic mechanisms (Doihara *et al.*, 2009). In an earlier study, TRPA1 mRNA was also detected in human and mouse mucosa (Purhonen *et al.*, 2008). Although functional data were obtained from the neuroendocrine cell line STC-1, here TRPA1 was suggested to play a role in intestinal cholecystokinin release.

Evidence also points to a role for TRPA1 in the intrinsic neurons of the ENS. TRPA1 immunoreactive fibres were found in the rat MP (Kondo *et al.*, 2009) and mRNA was detected in the isolated smooth muscle layer of rat small intestine (Nozawa *et al.*, 2009). TRPA1 agonists were furthermore shown to promote contraction of isolated strips of mouse colon (Penuelas *et al.*, 2007). However, mouse colonic compliance did not change by applying mustard oil or after TRPA1 deletion (Cattaruzza *et al.*, 2009).

TRPML and TRPP channels

The TRPML family contains three mammalian members (TRPML1–3). TRPML channels are expressed by intracellular endosomes and lysosomes and interact with a variety of vesicular proteins. Although functional studies suggest that TRPMLs play active roles in membrane fusion and fission, signal transduction and vesicular homeostasis, the exact mechanisms have not been elucidated (Cheng *et al.*, 2010).

The TRPP family was named after its founding member, polycystin kidney disease-2, a gene product mutated in many cases of autosomal dominant polycystic kidney disease and comprises three channel-like and five non-channel members (Delmas, 2005). They are believed to function independently or together as part of a multiprotein receptor/ion-channel complex, and are suggested to be involved in transducing Ca^{2+}-dependent mechanosensitive signals in response to cilia bending in renal epithelial and endodermally derived cells.

It is not known whether TRPML and TRPP channels are expressed in the GI tract. Consequently, relevance for a specific function for members of the TRPML and TRPP subfamilies in neurogastroenterology has not been reported.

Concluding remarks

Several animal and human studies have provided a vast amount of data indicating that TRP channels are present at almost all levels of GI control, yet their exact role remains rather elusive in most cases (Figure 2). Although it is clear that several TRP superfamily members play an important part in the control of GI motility, also here, mechanistic information about their *modus operandi* is still limited. The contribution of TRPC4 and TRPC6 in muscarinic receptor-induced contractility is quite well established while the role of TRPC1, TRPC2 and TRPA1 in intrinsic enteric neurons and the role of TRPC4 and TRPM7 in the generation of ICC pacemaker currents require further studies. Apart from motility control, TRP channels are also involved in epithelial functions. TRPM5 is, similar as in taste cells of the tongue, believed to play a role in taste signalling by EEC cells and brush cells in the gut. Serotonin release by EC cells and chlorhydropeptic secretion by parietal cells is suggested to be controlled by TRPA1 and TRPV1 respectively. The latter is furthermore proposed to play a role in acid sensing by esophageal epithelial cells. Because TRP channels are involved in many different aspects of sensory perception such as cold/heat, pressure/tension and pain, their role in sensation via visceral afferents and other sensory cell types present in the GI tract is not that surprising. Visceral mechano-, chemo-, thermo- and nociception are indeed shown to be mediated by TRPV1, TRPV4 and TRPA1 (and probably also TRPM8) channels. Importantly, in diseased states such as inflammation these channels are also implicated in visceral hypersensitivity and pain, sometimes even more pronounced than in healthy conditions.

Besides their sensitivity to physical and thermal stimuli, many of the TRP channels are sensitive to plant metabolites. Several of the noxious components that activate TRP channels are part of defence mechanisms that plants have developed against herbivores (Vriens *et al.*, 2008). Because many of these plant derivatives are present in food, GI cells become readily exposed. Spices like mint, mustard, pepper, cinnamon and garlic are known to affect TRP channels, which in most cases is likely to underlie their effects on the alimentary tract. A better understanding of TRP channel expression and function is essential to fully exploit the use of these simple food additives and comprehend their mechanism of action.

With respect to the regulation of GI motility, several TRP channels can be regarded as possible pharmaceutical targets as they are likely to modulate and gently tilt the balance to increase or decrease activity that is already endogenously present in the regulatory network of enteric neurons, ICC and smooth muscle cells. Reducing the increased sensory perception caused by visceral hypersensitivity in GI diseases such as IBD, IBS and FD is another area where TRP channels could be targeted with TRPV1 agonists the first ones being studied (Holzer, 2008b; Blackshaw *et al.*, 2010), and where the TRPV4 receptor may also be a target worth considering. Except for the agents targeting TRPV1, treatment of human GI diseases with TRP channel-related drugs is still in its infancy; therefore future studies are needed to specifically elucidate the pathophysiological role of TRP channels. As has been suggested in the case of TRPV1 (Holzer, 2008a), identifying the difference between such 'pathological' TRP channels and TRPs needed for normal GI physiology, will be crucial. Given the widespread expression and roles of TRP channels in both the gut and the rest of the body, specific targeting of these 'pathological' TRPs will be very challenging. In addition, the discovery of more specific and suitable ligands is warranted as well as a better understanding of the interaction with other signalling systems.

Indeed, TRP channel signalling is often clearly influenced by other signalling cascades. One such a signalling system already touched upon is the family of PARs. Proteases released from colonic biopsies of IBS patients have been shown to induce visceral hypersensitivity in mice via increased activity of PAR2 (Cenac *et al.*, 2007). An increasing amount of evidence indicates that these proteases induce hypersensitivity via the modulation of TRP channels. Especially, advances in the understanding of how PAR2 sensitizes TRPV1 brought up possible mechanisms by which inflammatory pain occurs. For example, the temperature of activation of TRPV1 is well below body temperature in the presence of PAR2 (Amadesi *et al.*, 2004) and in pulmonary vagal afferents, it has been shown that PAR2 increases the open probability of TRPV1 (Gu and Lee, 2009). Thus, PAR2 reduces the activation threshold for this receptor and therefore potentially leads to spontaneous pain sensation. A striking similarity, however, exists in how different TRP channels interact with PAR2. As apart from TRPV1, also TRPV4 and TRPA1 are suggested to interact with PAR2, interesting questions about this similarity have risen: Does PAR2-mediated sensitization of TRPA1 (Dai *et al.*, 2007) also involve an increase of TRPA1 open probability? Is the mechanism of action by which PAR2 interacts with TRPV4 similar? Answering these questions will not be trivial as some conflicting observations have been reported for instance in case of the TRPA1 and PAR2 interaction (Brierley *et al.*, 2009; Cattaruzza *et al.*, 2009).

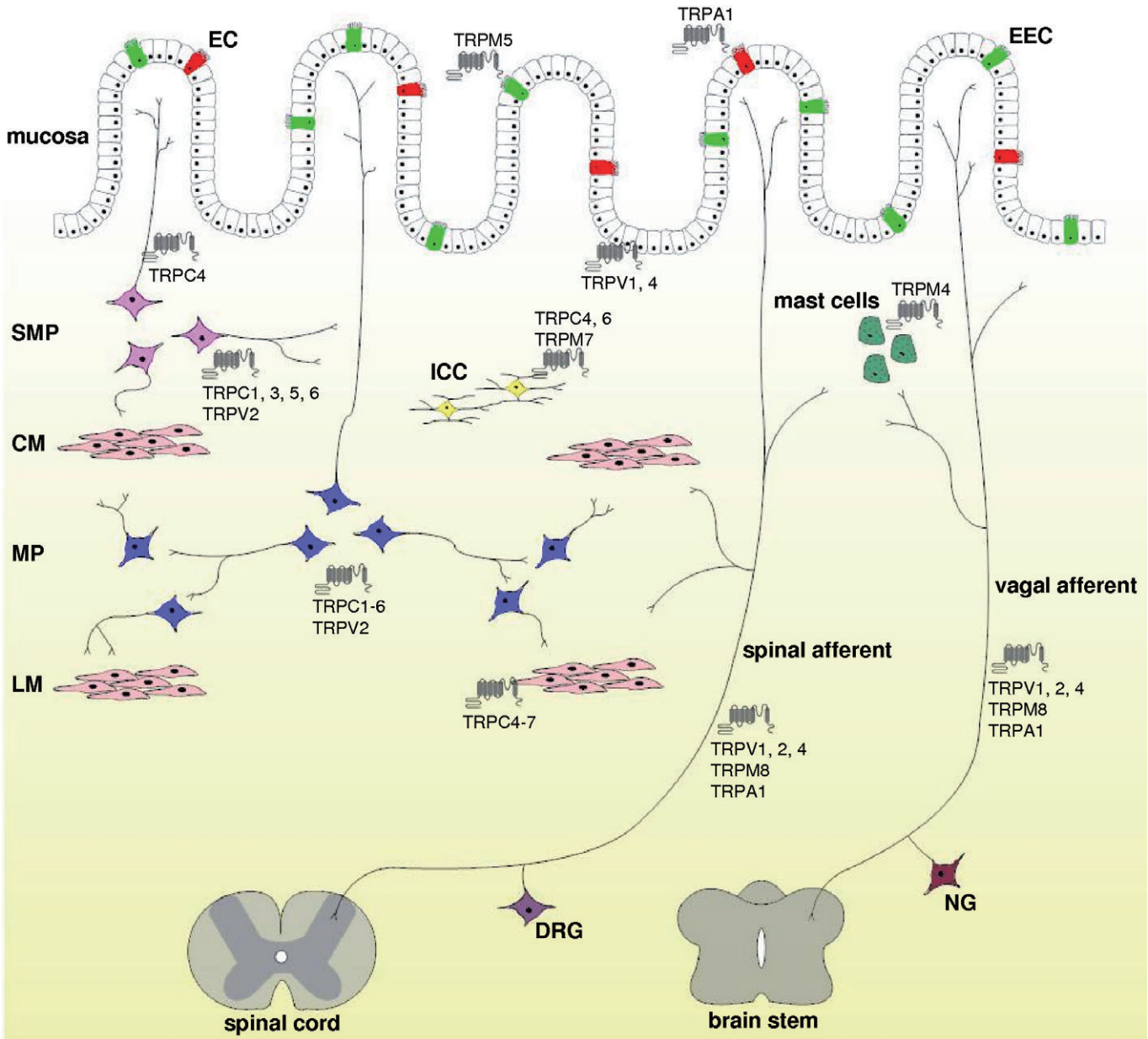

Figure 2

Schematic overview of transient receptor potential (TRP) channel distribution in the gastrointestinal (GI) tract. TRP channels can be found at almost all levels of GI control but their role remains rather elusive in most cases. In the GI mucosa, TRPs are expressed by enterocytes and different types of enteroendocrine (EEC, green) cells including enterochromaffin (EC, red) cells. They are involved in several epithelial functions such as nutrient sensing (TRPM5) and chlorhydropeptic secretion (TRPV1). Some TRP channels, especially TRPCs, are expressed by subtypes of enteric neurons in both the myenteric (MP, blue) and submucosal plexus (SMP, pink), yet knowledge about their function in the enteric nervous system is limited. Smooth muscle cells in the circular (CM) and longitudinal muscle (LM) layers also express TRPCs. More specifically, TRPC4 and TRPC6 play a role in muscarinic receptor-induced smooth muscle cell depolarization. Together with TRPM7, TRPC4 and TRPC6 are also implicated in the generation of pacemaker currents by interstitial cells of Cajal (ICC, yellow) but their exact function in the generation of slow waves is still debated. Apart from being involved in the intrinsic control of GI functions, a vast amount of data also points to a crucial role for TRP channels in visceral sensation by extrinsic afferents. Especially TRPs that are involved in different aspects of sensory perception such as temperature, pressure and pain are expressed by both spinal (purple) and vagal (maroon) afferents. TRPV1, 2, 4, TRPA1 and TRPM8 channels are shown to mediate visceral mechano-, chemo-, thermo- and nociception and are also implicated in visceral hypersensitivity in diseased states such as irritable bowel syndrome. The role of TRPM4 also deserves attention because factors released by these cells influence hypersensitivity to a large extent. For more details, see text.

Next to PAR2, there is also evidence that PAR4 is involved in the modulation of visceral nociception. This particular receptor would rather inhibit the PAR2-induced visceral hypersensitivity or down-regulate TRPV4 agonist-induced signalling. Because mustard oil-provoked visceral pain was reduced in PAR4-deficient mice, TRPA1 activity is probably also reduced by PAR4 (Auge *et al.*, 2009) but whether PAR4 likewise reduces the open probability of TRPV1 remains to be elucidated.

Although it is known that various TRP subunits can form heteromers (Schaefer, 2005), it is not clear whether certain pathological conditions induce particular heteromers to be formed. Functional interaction between different members of the TRP family could also be a possible mechanism via which visceral pain is mediated.

Overall, we conclude that the presence of TRP channels at almost all levels of GI control points at their involvement in gut homeostasis. Several TRP superfamily members play an important part in the control of GI motility and visceral sensation. They are also implicated in visceral hypersensitivity and pain but the exact role of most of them still remains rather elusive. Hence, it is obvious that TRP channels can be regarded as new targets for pharmacological intervention in the treatment of GI diseases. However, a substantial amount of work is still necessary to fully understand their mechanism of action. Nonetheless, thanks to the increasing number of publications in this field, as illustrated in this review, we consider this possible in the near future.

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Statement of conflict of interest

The authors have nothing to declare.

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