The transient receptor potential channel TRPA1: from gene to pathophysiology

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Received: 4 September 2012 / Revised: 6 September 2012 / Accepted: 6 September 2012
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Abstract The Transient Receptor Potential Ankyrin 1 channel (TRPA1), is a member of the large TRP family of ion channels, and functions as a Ca\textsuperscript{2+} permeable non-selective cation channel in many different cell processes, ranging from sensory to homeostatic tasks. TRPA1 is highly conserved across the animal kingdom. The only mammalian TRP subfamily member, TRPA1, is widely expressed in neuronal (e.g. sensory dorsal root and trigeminal ganglia neurons)- and in non-neuronal cells (e.g. epithelial cells, hair cells). It exhibits 14–19 amino-(N-)terminal ankyrin repeats, an unusual structural feature. The TRPA1 channel is activated by noxious cold (<17 °C) as well as by a plethora of chemical compounds that includes not only electrophilic compounds and oxidants that can modify, in an alkylative or oxidative fashion, nucleophilic cysteine residues in the channel’s N-terminus, but also compounds that do not covalently bind to the channel proteins (e.g. menthol, nifedipin). Based on localization and functional properties, TRPA1 is considered a key player in acute and chronic (neuropathic) pain and inflammation. Moreover, its role in the (patho)physiology of nearly all organ systems is anticipated, and will be discussed along with the potential of TRPA1 as a drug target for the management of various pathological conditions.

Keywords Transient receptor potential · Ca\textsuperscript{2+} channels · Ankyrin repeat domain · Nociception · Mechano-sensing · Channelopathies

Introduction

The superfamily of Transient Receptor Potential (TRP) ion channels comprises unique sensory proteins that are expressed in almost every tissue and cell type, and that play an important role in diverse homeostatic functions. TRP channels are organized into seven subfamilies: the TRPC (‘Canonical’), TRPV (‘Vanilloid’), TRPM (‘Melastatin’), TRPP (‘Polycystin’), TRPML (‘Mucolipin’), the TRPA (‘Ankyrin’), and TRPN (‘NOMP-C’) (for reviews see [58, 100, 210, 304]). One member of the TRP family, the ankyrin-repeat channel TRPA1 is of special interest for its functional diversity as a sensor of irritating and cell damaging signals, and its important role in many different diseases. This review aims at providing a comprehensive review of our current knowledge on structure, function and druggability of this fascinating ion channel.

The \textit{trpa1} gene

The \textit{trpa1} gene was originally cloned from lung fibroblasts in 1999 [128]. It was originally baptized ANKTM1 as it contains multiple ankyrin repeats in the amino-(N-)terminal part [260]. The human \textit{trpa1} gene is composed of 27 exons and spans 55,701 base pairs (bp) of the human chromosome 8q13. It has been also found in many vertebrates and invertebrates, including mouse, rat dog, chicken, zebrafish, fruit fly and \textit{Caenorhabditis elegans}. In contrast to mammals that contain only one \textit{trpa1} gene, other classes of the Animalia Kingdom contain multiple TRPA1 homologues (e.g. 4 in
fruit fly, 2 in C.elegans, 2 in zebrafish, 4 in sea squirt Ciona intestinalis). It is believed that all animals contain at least one trpa1 gene.

There is an evolutionary link among electrophile-sensitive TRPA1s across species. Interestingly, the phylogenetic trees of TRPA proteins indicated a clear separation of electrophile-sensitive TRPA1s and electrophile-insensitive (or basal) TRPAs. All electrophile-sensitive TRPA1s derive from a common ancestor of both vertebrates and invertebrates. Therefore, it can be considered that the function of TRPA1 has been conserved for ~500 millions of years [133, 175] (Fig. 1).

### Protein structure

The trpa1 gene encodes a ~1,100 amino acid (aa) protein of ~120–130 kDa (e.g. 1,119 aa in human, 1,125 aa in rat, 1,115 aa in mouse, 1,120 aa in zebrafish, 1,197 aa in fruit fly, 1,193 aa in C. elegans). In addition to full length protein versions, shorter splice variants have also been identified. Similarly to other TRP channels, TRPA1 very likely functions as a homotetramer composed of TRPA1 proteins [208, 211]. (see also Fig. 2).

The predicted structural topology of TRPA1 is similar to other TRP proteins and comprises six putative transmembrane segments (S1-6). The putative ion permeable site (pore, pore helix and selectivity filter) is located between S5-S6 and the first extracellular loop between S1-S2 contains 2 putative Asn-glycosylation sites. The long N-terminal moiety contains up to 18 ankyrin repeat domains (ARDs) composed of 33 aa that might be involved in protein-protein interactions as well as in channel trafficking to the plasma membrane. Deletions of TRPA1 ARDs were shown, in fact, to negatively affect the insertion of the channel into the plasma membrane [211]. Recently, it has been shown in a model of TRPA1 with 17 ankyrin repeats (AR), that Ca\(^2+\) binding to the ARD changes the overall structure of the N-terminus, increasing its stiffness and diminishing its end-to-end distance. In the transmembrane domain of TRPA1, N855, the important residue close to TM5 and associated with familial episodic pain syndrome, forms a strong link between the S4-S5 connecting helix and S1, thereby creating a direct force link between the N-terminus and the gate [318].

The putative EF hand motive involved in intracellular Ca\(^{2+}\) ([Ca\(^{2+}\)]\(_i\))-dependent activation of TRPA1 is located between ARD11 and ARD12 [72, 325]. The importance of this EF hand site is questionable, since point mutations in this region have only modest effects on [Ca\(^{2+}\)]\(_i\)-dependent activation mechanism, while deletions impair trafficking of the truncated channel to the plasma membrane [211].

Another putative Ca\(^{2+}\)-binding domain is composed of a cluster of acidic residues in the distal carboxy-(C)-terminus of TRPA1 [265]. Four conserved residues in human TRPA1, Glu1077, Asp1080, Asp1081 and Asp1082, have strong effects on the Ca\(^{2+}\) - and voltage-dependent potentiation and/or inactivation of agonist-induced responses. Truncation of the C-terminus by only 20 residues selectively slowed down the Ca\(^{2+}\)-dependent inactivation without affecting other functional parameters. This acid cluster, which shows partial homology to the Ca binding bowl in BKCa channels, may represent the long-sought Ca\(^{2+}\)-sensing domain [265].

The only direct structural insights on TRPA1 channel are those available from a 16 Å resolution structure of purified, amphipol-stabilized,TRPA1 proteins analyzed by single-particle electron microscopy (EM) [62]. This structural model suggests that the critical N-terminal cysteine residues involved in electrophilic activation are located at the interface between neighboring subunits and form a ligand-binding pocket, allowing disulfide bonding between the cysteine residues [290]. Covalent modifications by thiol-reactive
compounds within such pockets may alter interactions between subunits and promote conformational changes that translate to modification of the gating mechanism [62, 290].

TRPA1 expression

Originally, TRPA1 was described as a nociceptive channel expressed in sensory neurons of dorsal root ganglia (DRG), trigeminal ganglia (TG), and nodose ganglia and responding to noxious cold and pungent compounds, as well as a putative transduction channel found in the inner ear (the organ of Corti) and involved in hearing [24, 197, 260]. However, growing evidence suggests that TRPA1 is a widely expressed channel present in many organs and tissues, including brain, heart, small intestine, lung, skeletal muscle, and pancreas [259].

Sensory neurons, sensory and peripheral nervous systems

In TG, TRPA1 is expressed in unmyelinated (C) and small myelinated (Aδ) axons, with only occasional identification in large myelinated axons. More than 25% of TRPA1 containing neurons are peptidergic and release substance P and calcitonin gene-related peptide (CGRP), while the remaining neurons were identified as non-peptidergic neurons by isolectin B4 (IB4; ~45%). Trigeminal sensory nuclei (TSN) and the spinal dorsal horn (DH) are immunopositive for TRPA1. High expression can be found in terminals of the superficial laminae of the trigeminal caudal nucleus (Vc) and DH. Neurons in Vc respond to intraoral cooling and TRPA1 agonists, receiving inputs from TRPM8, TRPA1 and/or TRPV1-containing primary trigeminal afferents [85, 317].

The TRPA1 terminals, characterized by clear round vesicles, are presynaptic to one or two dendrites, with a small degree of synaptic divergence and little presynaptic modulation. Occasionally, TRPA1 is also observed in postsynaptic dendrites [143]. In the spinal cord, the substantia gelatinosa (SG) is a key site for integration of noxious inputs. TRPA1 is involved in the spontaneous glutamatergic excitatory transmission in substantia gelatinosa (SG; lamina II) [124]. Presynaptically located TRPA1 channels increase glutamate release, triggering synaptic transmission onto SG neurons and evoking excitatory postsynaptic potentials (EPSCs) in vertical- and radial-, but not islet or central SG cells [284]. TRPA1 is involved in the presynaptic glyciner-gic neurotransmission in the dorsal horn [54]. Moreover, TRPA1 is also localized presynaptically in terminals of primary afferents that innervate spinal inhibitory gamma-aminobutyric acid (GABA) neurons, which make contacts with SG neurons [155].
TRPA1 is also expressed in the autonomic nervous system. Sympathetic superior cervical ganglia (SCG) contain a population of cold sensitive neurons that express TRPA1 [256]. TRPA1 is also present in the majority of the nodose (placodes-derived) and jugular (neural crest-derived) esophageal nociceptors [42] that sense esophageal distention and respond to chemical perfusion. Bradykinin activates most nodose and all jugular C-fibers via a TRPA1 dependent mechanism [150, 315].

In vagal fibers innervating the heart, TRPA1 contributes to vasovagal reflexes [230], whereas TRPA1 activation in vagal sensory nerves innervating the airways provides responses to environmental stimuli, eliciting action potential discharge in airway afferent C-fibers and the consequent nocifensor reflexes [202, 279]. Similarly, nasal TG nerve endings are particularly sensitive to oxidants formed in polluted air and during oxidative stress as well as to chlorine that is frequently released in industrial and domestic accidents [30]. In general, TRPA1 is primarily expressed in small-diameter, nociceptive neurons of primary sensory afferent nerves innervating the whole respiratory tract [9, 26, 29, 324].

TRPA1 is highly expressed in neurons of the pelvic nerve (PN) that innervate colon. Axons of these neurons arise from DRG at thoracolumbar (TL) and lumbosacral (LS) spinal levels [162]. A similar expression level was also observed in inhibitory enteric neurons in the cecum and the colon. Inhibitory motoneurons and descending interneurons, cholinergic neurons, and intrinsic primary afferent neurons display lower TRPA1 expression levels. The activation of TRPA1 in these neurons inhibits contractility in the colon but not in the stomach or the small intestine and is involved in inhibition of spontaneous neurogenic contractions [229].

In primary sensory neurons of the lumbosacral plexus innervating the bladder (DRG level L6-S1 DRG), TRPA1 expression is linked with the bladder afferent transduction, causing hyperreflexia through C-fiber-mediated afferent pathway [6, 76].

In prostate, TRPA1 is located on nerves that are positive for CB1, CB2, CGRP, nitric oxide synthase (NOS), or vesicular acetylcholine transporter (VAcHt). It has been proposed that TRPA1 together with CB receptors may function as mediator of mechanooafferent signals, epithelial homeostasis, emission, or inflammation of the human prostate [108].

Central nervous system

The TRPA1 expression is clearly demonstrated in the central nervous system (CNS). In the hippocampus, TRPA1 is linked with activation of the cannabinoid receptor CB1 upon hippocampal formation [147]. It is also expressed in neurons of the nucleus supraopticus. TRPA1, most likely together with TRPV1, is involved in excitatory synaptic inputs to the magnocellular neurosecretory cells (MNCs) that produce vasopressin in the supraoptic nucleus (SON). TRPA1 exists at presynaptic terminals to the MNCs and enhances glutamate release in the SON [312].

In the brain stem, TRPA1 is expressed on visceral afferent pathway and regulates glutamate release. The TRPA1 agonists, such as allylisothiocyanate (AITC), effect mechanically dispersed nucleus tractus solitarii neurons (~60 % neurons), suggesting non-nociceptive responses related to TRPA1 [262].

In astrocytes, which contribute to the formation and function of synapses, TRPA1 expression might be linked with regulation of inhibitory synapses. TRPA1 activation evokes frequent and highly localized ‘spotty’ Ca2+ membrane-near microdomains. The reduction of astrocyte resting Ca2+ concentrations mediated by TRPA1 channels decreases the interneuron inhibitory synapse efficacy by impaired GABA transport, resulting in elevated extracellular GABA contents [252].

Inner ear

The unique ARD structure of TRPA1 hinted that it might possibly be a mechanosensitive channel in the inner ear [60]. TRPA1 is expressed in the the stria vasularis, the organ of Corti and outer and inner hair cells (OHCs and IHCs) of the cochlea [258, 272].

Cardiovascular system

In vascular endothelial cells, TRPA1 function is related to endothelium-derived hyperpolarizing factor (EDHF) responses [80, 263]. TRPA1 is activated by environmental irritants, pungent compounds found in foods such as garlic, mustard, and cinnamon, as well as metabolites produced during oxidative stress. TRPA1 agonists cause arterial dilation through two distinctive pathways. TRPA1 channels expressed on perivascular nerves mediate vasodilatation of peripheral and cerebral arteries in response to chemical agonists through release of CGRP. In the endothelium, TRPA1 is mainly concentrated within myoendothelial junction sites. Its activation causes endothelium-dependent smooth muscle cell hyperpolarization and vasodilatation that requires the activity of small and intermediate conductance Ca2+-activated K+ channels [79, 233].

Expression of TRPA1 in the pancreas

TRPA1 is abundantly expressed in rat pancreatic islets [45]. Allylisothiocyanate and 15-deoxy-Delta[12, 14]-prostaglandin J2 (15dPGJ2), significantly induced Ca2+ influx in β cells, and the specific TRPA1 inhibitors block the effects elicited by electrophilic activation [213].
Gastrointestinal tract

In the small intestinal and colonic mucosa, TRPA1 functions together with intestinal odorant receptors (ORs). As the gut lumen is continually exposed to a great variety of agents, including noxious compounds, TRPA1 acts as a chemosensor that detects the luminal environment and modulates gastrointestinal functions. Stimulation of ORs modulates epithelial permeability and electrogenic anion secretion in human and rat colon which is most likely mediated by activation of TRPA1 channels [130]. A relatively high expression of TRPA1 is also observed in enterochromaffin cells throughout the GI tract. It has been shown that TRPA1 agonists delay in vivo gastric emptying through serotonergic pathways [73].

Respiratory system

TRPA1 is highly expressed in most nonneuronal cell types forming the pulmonary system where it plays a fundamental role not in normal airway function but becomes extremely important for acquired diseases [1, 44]. It has been shown that activation of TRPA1 might modulate the chemokine release in inflamed airways [196].

Skin

In nonneuronal skin cells, such as human keratinocytes and fibroblasts, activation of TRPA1 mediates secretion pattern of the eicosanoids, such as prostaglandin E2 PGE2 and leukotriene B4 (LTB4) and provokes a long-lasting local erythema [127]. In the basal layer of the epidermis, TRPA1 is also co-localized with the melanocyte marker, pMel-17. It has been demonstrated that in melanocytes TRPA1 affects the expression of proinflammatory cytokines, including interleukin-1α and β (IL-1α, IL-β) [12].

Dental pulp

In human teeth, TRPA1 is highly expressed in dental pulp fibroblasts where it might be involved in cold responses and pain associated with mechanotransduction [83]. The majority of pulpal afferents also express TRPA1, and therefore TRPA1 might be considered an important target for the treatment of dental sensitivity [43, 116, 141].

Stem cells

Neural crest-like stem cells from neonatal mouse epidermis can be converted to Schwann precursor cells, pigmented melanocytes, chondrocytes, or functional sensory neurons showing voltage-gated sodium channels and TRPA1 [266].

Cellular distribution and turnover of TRPA1

TRPA1-mediated nocifensive behavior can be sensitized in vivo via protein kinase A (PKA)/phospholipase C (PLC) signaling and/or application of TRPA1 agonists such as mustard oil (MO). Since both stimuli increase TRPA1 expression in the plasma membrane and application of MO enlarges cell capacitance, one possible mechanism might be linked with increased exocytosis in these cells. In line with this hypothesis, tetanus toxin attenuates the response to the second of two pulses of MO in neurons, suggesting that vesicle fusion increases functional TRPA1 expression at the plasma membrane. Thus, translocation of TRPA1 to the plasma membrane might represent one of the mechanisms controlling TRPA1 functionality upon acute activation or inflammatory signals [248].

The cellular levels of TRPA1 seem to be controlled by a potential post-translational mechanism related to the human tumor suppressor CYLD. CYLD is an ubiquitin hydrolase that binds to TRPA1 and de-ubiquinates the channel. De-ubiquitination causes increased cellular levels of TRPA1 proteins. Thus, oncogenic mutations of CYLD might alter TRPA1-mediated responses by rendering the channel susceptible to ubiquitinylation [259]. Thus, association of TRPA1 with enzymes of the ubiquitin pathway might be important mechanisms that regulate cellular levels of TRPA1.

Biophysical properties of TRPA1

The pore and single channel properties

The constitutive open TRPA1 channel evokes outwardly rectifying currents (Aa, C) that rapidly inactivate at positive potentials (Bb,c). The nature of this inactivation is still unknown. The activation of TRPA1 by electrophilic compounds results in large inward currents whereas the outward rectification is mostly abolished (Bb). In contrast, TRPA1 activation with non-electrophilic compounds still displays outward rectification. The reason for these differences still has to be elucidated. Extracellular Ca²⁺ ([Ca²⁺]e) level has an effect on TRPA1 currents. In the presence of [Ca²⁺]e activated TRPA1 currents decline rapidly (decay, desensitization C) whereas in its absence, both current activation as well as current decay, are delayed (Fig. 3).

As for other TRP channels, the pore of TRPA1 is formed by the selectivity filter and the pore helix of the four subunits in the tetrameric channel. Based on the relative permeability of the nonstimulated channel to cations of different size, the TRPA1 pore has a diameter of approximately 11 Å. It is also predicted that binding of Ca²⁺ in the pore may hinder mono-valent cation permeation, resulting in a large fractional Ca²⁺ current. Approximately 17 % of the inward TRPA1 current is
carried by Ca\(^{2+}\). This surprisingly high fractional Ca\(^{2+}\) current places TRPA1 as the third most Ca\(^{2+}\) permeable TRP channel (after TRPV5 and TRPV6).

The residue Asp918 seems to play a decisive role in determining the Ca\(^{2+}\) permeation through this channel [136, 211]. Another residue, Glu923, likely located in the outer pore turret, seems to create a Ca\(^{2+}\) microdomain at the pore entry [33]. Since TRPA1 seems to be partially a constitutive open channel, sustained and increased levels of endogenous TRPA1 agonists might be present especially in various pathophysiological conditions [149].

In outside-out patch recordings, using N-methyl-D-glutamine (NMDG\(^{+}\)) as the sole external cation and Na\(^{+}\) as the internal cation, TRPA1 activation results in dynamic changes in permeability to NMDG\(^{+}\) [52, 295]. This is due to a progressive but reversible pore dilation process that also causes an increase in divalent cation selectivity and fractional Ca\(^{2+}\) current [19]. Mutations of the critical pore residue Asp918 reduces the divalent permeability and fractional Ca\(^{2+}\) current but also prevents MO-induced increases in Ca\(^{2+}\) permeation [136]. Obviously, TRPA1 can exist in at least two distinct open states: a restricted and a dilated state. The restricted state is a non-selective cation channel, whereas the dilated state allows influx of much larger molecules, e.g. Yo-Pro (Mw ~630) and NMDG. It is under discussion whether the dilated state is regulated by extracellular calcium. Amiloride and its analogue 5-(N,N-Dimethyl)amiloride (DMA) block the channels but in the dilated state these open channel blockers penetrate deeper
into the pore providing a more efficient block [18]. It is very likely that the pore region is involved in channel gating. Residues within the S6 inner pore-forming region of human TRPA1 contribute to gating by electrophiles in a voltage-dependent manner. The alanine substitution in the conserved mid-S6 Pro949 (Pro949Ala) strongly affects the activation/deactivation and ion permeation. The proline 949 is structurally required for the normal functioning of the TRPA1 channel. Another mutation, Asn954Ala, generates a constitutively open channel, suggesting a role in stabilizing the closed conformation. Alanine substitutions in the distal GlyXXXGly-motif decrease the relative permeability of the channel for Ca2+ and strongly affected its activation/deactivation properties, indicating that the distal Gly 962 stabilizes the open conformation. The glycine 958, on the other hand, provides additional tuning leading to decreased channel activity. The inner pore region probably controls conformational changes required for transitions between open and closed states of the TRPA1 channel [27].

In contrast to TRPA1 pore properties that change with different activation modes, single channel properties depend on the experimental condition. In divalent cation-free solution, TRPA1 has a single channel conductance of ~112 pS at negative and positive potentials. In the presence of extracellular Ca2+ and Mg2+, the conductance at negative potentials (inward direction) is reduced to 55–65 pS, depending on the concentration of the divalent cations. TRPA1 has a rather high Ca2+ selectivity, P_Ca/P_Na is ~6 for the constitutive open channel and ~9 for the channel activated with electrophilic agonist. The fractional Ca2+ currents are respectively ~17 % for the constitutively open and 23 % for the agonist activated channel [136]. The magnesium ion blocks the open channel but is still permeable at negative potentials \((P_{Mg}/P_{Na} \approx 2, P_{Ba}/P_{Na} \approx 3.5)\). Relative inorganic cation permeabilities are \(Li^+ >Na^+ >K^+ = Rb^+ >Cs^+ \) (1.2 : 1 : 0.98 : 0.98 : 0.95), indicating a strong field strength binding cation site in the channel (Eisenman XI). Organic cations permeate with the sequence of \(Na^+ \sim \text{Dimethylamine} > \text{Trimethylamine} > \text{Tetramethylammonium} > \text{N,N,N,N-tetramethylammonium} \) and \(\text{NMDG}^+ \) (1 : 0.99 : 0.7 : 0.4 : 0.1). Electrophilic agonists increase the permeability to large organic cations due to a pore dilation of ~1 to 3 Å [33, 136].

Single channel properties of TRPA1 seem to be also regulated by co-expression with TRPV1. Very likely, TRPA1 and TRPV1 proteins form a complex that can influence single-channel currents through TRPA1. Co-expression of TRPV1 with TRPA1 results in outward rectification of single-channel current–voltage relationships (I-V) and substantial modulation of the open probability at negative holding potentials. TRPV1 does not influence the single channel conductance in [Ca2+]o-free solution. Thus, single-channel properties of TRPA1 are regulated by TRPV1 independently of intracellular Ca2+ but rather by direct interaction [257].

Structure—function relationship in channel gating

TRPA1 is regulated by negatively charged ligands such as phosphoinositides or inorganic polyphosphates, most likely through an interaction with as yet unidentified positively charged domain(s) in the cytoplasmic tails. The positively charged residues in the C-terminal tail of TRPA1, Lys969, Arg975, Lys988 and Lys989, are involved in electrophilic agonist and voltage-dependent gatings. Another positively charged region is centered around Lys1048 and Lys1052. Single alanine mutations in this region completely abolished agonist and voltage-dependent activation. In the distal portion of the C-terminus, the alanine substitutions at Lys1092 and Arg1099 reduce the channel sensitivity to electrophilic agonists, and increased the voltage-induced steady-state responses. Thus, a stretch of basic residues in the C terminus represent possible interaction sites for negatively charged molecules that are generally considered to modulate TRPA1 [244].

Modulation and regulation of TRPA1

TRPA1 is considered as a very attractive target for development of analgesic and anti-inflammatory drugs. Therefore, the pharmacology of the TRPA1 channel is of huge importance. Unfortunately, striking differences between human and rodent TRPA1 homologues complicate TRPA1-targeted drug discovery. Many compounds that show antagonistic effect with human TRPA1 can behave as agonists or show no activity when examined in rat and mouse. Thus, functional differences have to be taken carefully into account when the modulation of this channel is considered (see also [31]).

Electrophilic activators

TRPA1 has a remarkable gating promiscuity. Electrophilic TRPA1 ligands of environmental-, dietary- or endogenous origin modify nucleophilic cysteine and lysine residue(s) in the N-terminus of the channel [15]. Chemical activators of TRPA1 are structurally as diverse as their source: isothiocyanates (the pungent compounds in MO, wasabi, and horseradish) [14, 129], methyl salicylate (in winter green oil) [14], cinnamaldehyde (in cinnamon) [14] allicin and diallyl disulphide (in garlic) [23, 174], acrolein (an irritant in vehicle exhaust fumes and tear gas) [23] and Δ9 tetrahydrocannabinol (Δ9THC, the psychoactive compound in marijuana [129, 219, 260].

Allyl isothiocyanate from MO is one of the most efficient electrophilic activators of TRPA1. In human TRPA1, sulf-hydryl reacting agents modify cysteines Cys619, Cys639 and Cys663 located between the last ARD and S1 [119].
In the mouse TRPA1 homologue the most reactive cysteine residues are Cys415 and Cys422 between 10th and 11th ARDs and, similarly to the human version, Cys622 between last AR and S1 [173] (for a review see [47]) (for a review see [225]). Other electrophiles, such as cinnamaldehyde (CA), super CA (SC), SC –alkyne (SCA), acrolein, 2-pentenal, MO, alkyn (MOA), iodoacetamide (IA), IA-alkyne (IAA), and 2-(trimethylammonium)ethyl methanethiosulfonate bromide (MTSEA; used for cysteine scanning), are capable of reacting with cysteine residues and act as TRPA1 activators. Although it is now generally accepted that TRPA1 is activated through covalent modification of specific cysteines, the precise mechanism and the chemistry of this covalent modification with unsaturated carbonyl-containing compounds is unclear. Channel activation occurs with chemicals that react with cysteine residues via alkylative conjugate addition [241], but unravelling of the molecular details underlying activation and deactivation of TRPA1 via covalent modifications still remains an exciting challenge.

Importantly, TRPA1 is a sensor for oxygen. O2 sensing is based upon disparate processes. Prolyl hydroxylases (PHDs) exert oxygen-dependent inhibition on TRPA1 activity in normoxia. However, a direct O2 action overrides the inhibition via the high sensitivity of TRPA1 to cysteine-mediated oxidation in hyperoxia. Therefore, TRPA1 is activated through relief from the PHD-mediated inhibition in hypoxia. TRPA1 in the vagal and sensory neurons is therefore a critical sensor of both hyperoxia and hypoxia which will both enhance vagal discharges. TRPA1 is thus an exciting new player in O2-sensing [269]. In addition, the hypoxia inducible factor-1α (HIF1α) is also coupled with the expression of TRPA1 [113].

In general, reactive oxygen species (ROS) that cause cysteine oxidation or disulfide formation, reactive nitrogen species (RNS) like nitric oxide (NO) that mediate S-nitrosylation, and reactive carbonyl species (RCS), like electrophilic prostaglandins (PG) and α/β unsaturated aldehydes that alkylatively modify cysteine, are all potential TRPA1 activators [270, 271, 280].

It is widely known that inhalation of ozone is a major health risk in industrialized nations, impairing lung function through sensory neural-mediated pathways (vagal nociceptive C type bronchopulmonary nerves). Ozone can stimulate TRPA1 but has no effect on TRPV1 [278]. Hydrogen peroxide (H2O2), a common industrial and household chemical, is also generated within cells, causing pain sensation via activation of TRPA1. Effects of H2O2 can be mimicked by other ROS as well as by RNS. Cysteine-reducing agents, such as dithiothreitol (DTT), suppress H2O2-induced TRPA1 activation, whereas cysteine-oxidizing agents activated TRPA1 [247]. Oxygen (O2) is a prerequisite for cellular respiration in aerobic organisms but also elicits toxicity. TRPA1 senses O2 in mechanistically distinct ways: prolyl hydroxylases (PHDs) exert O2-dependent inhibition on TRPA1 but direct O2 action of TRPA1 can override this inhibition, and, in hypoxia TRPA1 is activated through relief from the PHD-mediated inhibition [269].

TRPA1 confers a sensitivity towards near ultraviolet (UVa) light. UVA light activates TRPA1 currents in a wavelength-dependent and membrane-delimited manner. Light-induced TRPA1 activation is caused by highly ROSs and provides an additional mode of activation, which renders TRPA1 a likely molecular candidate in processes leading to painful or burning sensations during photodynamic therapy or upon local application of hydrogen peroxide [118].

Among RNS, NO is a most potent TRPA1 activator. It can induce acute pain in humans and plays an important role in pain sensitization caused by inflammation and injury in animal models. NO acts both in the central nervous system via a cyclic GMP pathway and in the periphery on sensory neurons through direct activation of TRPA1 (and also TRPV1). The endothelial tetrahydrobiopterin (BH4), an essential co-factor for NO production, causes activation of a subset of DRG neurons by TRPA1 activation [191]. Nitric acid (9-0A-NO2), an electrophilic fatty acid byproduct of NO and nitrite reactions is also a potent TRPA1 activator. Excessive nitric oxide during inflammation (nitrative stress), leads to the nitration of phospholipids resulting in the formation of this highly reactive cysteine modifying agent. 9-0A-NO2 failed to activate TRPA1 in which the cysteines at position 619, 639 and 663 and the lysine at 708 had been mutated (the TRPA1-3C/K-Q mutant) [274]. The effects of OA-NO2 are blocked by DTT but could not be prevented or reversed by an NO-scavenger carboxy-PTIO [250].

Another important RNS, hydrogen sulfide (H2S) is a malodorous gas that functions as an endogenous gasotransmitter in humans and is involved in a wide variety of processes including nociceptive processes [192]. H2S evokes CGRP release from sensory neurons of isolated rat tracheae, increasing microcirculation in several organs, and TRPA1 receptor activation might be a major mechanism underlying the vasoreactive effects of H2S [231]. Neurogenic inflammation, hyperalgesia and pain caused by H2S are all enhanced under acidic conditions [214]. Hydrogen sulfide increases cAMP levels in neuronal and glial cell lines and primary neuron cultures. Hydrogen sulfide-induced [Ca2+]i responses are inhibited by the reducing agent DTT. [214]. Beside effects on TRPA1, H2S may be involved in multiple signaling pathways and produce various effects on ion channels such as T-type calcium channels and ATP-sensitive K+ (KATP) channels, which may inhibit or promote nociception. T-type Ca2+ channel blockers together with TRPA1 channel blockers efficiently attenuate NaHS/H2S-induced mechanical hyperalgesia and allodynia in rodents [215].
High concentrations of carbon dioxide (CO₂), as found in carbonated beverages, evoke a mixture of sensations, including a stinging or pungent quality. The stinging sensation originates from activation of TG nociceptors that express TRPA1 and innervate the respiratory, nasal, and oral epithelia. Carbon dioxide diffuses into cells and produce intracellular acidification thereby gating TRPA1. Consistent with this mechanism, TRPA1 is activated in a dose-dependent manner by intracellular protons [288]. Acetic acid produces an irritating sensation that can be attributed to activation of TRPA1 nociceptors. Other weak organic acids, including propionic, formic, and lactic acid, but not strong acids, also activate TRPA1. Importantly, preactivation of TRPA1 channels to weak acids [99, 287]. Alkaline pH also causes pain via activation of TRPA1. Two N-terminal residues, Cys422 and Cys622, are responsible for high pH perception. Pain behaviors evoked by intraplantar injection of ammonium chloride are completely reduced in TRPA1 ko mice [96].

Cyclopentenone PGs, 15dPGJ2, PGA2, and PGA1, formed by dehydration of their respective parent PGs, PGD2, PGE2, and PGE1, possess a highly reactive α, β-unsaturated carbonyl group and can gate TRPA1. Thus, 15dPGJ2 activates TRPA1 expressed in HEK cells as well as in mouse TG neurons [61]. This effect was not mimicked by their non-electrophilic precursors, PGE2, an aldol, PGD2, a saturated cyclopentanone, or PGB2 a β, β-disubstituted enone, where the electrophilic element is lacking or is not reactive due to steric hindrance. Cyclopentenone PGs produce pain by direct stimulation of nociceptors via TRPA1 activation, and are proalgesic. Thus, preventing TRPA1-dependent nociceptor activation by cyclopentenone PGs with TRPA1 antagonism may suppress pain without the adverse effects of cyclooxygenase inhibitors [184], that act upstream and less selectively in the PG cascade, by inhibiting cyclooxygenases.

Tissue damage release factors, such as 4-hydroxynonenal (4-HNE) and another endogenously-produced alkenal, 4-oxononenal, are potent TRPA1 activators [276]. 4-Hydroxynonenal is an α, β-unsaturated hydroxyalkenal which is produced in inflamed tissues during peroxidation of membrane phospholipids by ROS. It evokes release of substance P and CGRP from nerve endings, causing extravasations of plasma proteins into the surrounding tissue. The activation of TRPA1 with 4-HNE promotes acute pain, neuropeptide release and neurogenic inflammation. 4-Hydroxynonenal acts via covalent modification of the cysteine/lysine residues in the TRPA1 NH₂-terminus since the TRPA1-3C/K-Q mutant is insensitive to 4-HNE [281]. Effects of endogenous 4-HNE are abolished with DTT, suggesting that they are mediated by a redox process. In contrast, the action of the alkenyl aldehydes and 15d-PGJ2 is not reversed by DTT, suggesting that these agents form irreversible Michael adducts [3].

Apart from naturally occurring chemical activators, TRPA1 is also sensitive to a host of compounds used in every aspect of modern life, including military use. Constituents of tear gases such as methyl isocyanate, 1 H-dibenzo[β, e]azepines (morphanthridines) and dibenz[b,f][1,4]oxazepines, mediate TRPA1 activation [104]. The release of methyl isocyanate in Bhopal, India, caused the worst industrial accident in history [29]. Toluene diisocyanate (TDI) is used as a chemical intermediate in the production of polyurethane polymers such as foams, coatings, and elastomers. It is a reactive hazardous irritant, causing respiratory symptoms such as cough, rhinitis, dyspnea and chest tightness in exposed workers [275]. Formaldehyde and its water solution, formalin, directly activate TRPA1 and are widely used in animal models for testing analgesic compounds [188]. Painful responses to iodoacetamide, a nonspecific cysteine-alkylating agent, are due to TRPA1 activation [176]. TRPA1 is also activated by disulfiram (Antabuse), a compound used in the treatment of alcohol abuse, and by the anti-fungal agent, chlordantoin [177].

Non-electrophilic modulators

Beside the huge number of electrophilic activators, TRPA1 can also be modulated by other compounds that are unlikely to induce covalent modifications of the channel proteins.

In general, anesthetic agents can induce a paradoxical activation and sensitization of TRPA1. Propofol (2,6-diisopropylphenol), a commonly used intravenous anesthetic, elicits intense pain upon injection. TRPA1 is a key molecule for propofol-induced excitation of sensory neurons [92]. Propofol’s effects on sensory neurons may be clinically important and may contribute to peripheral sensitization to nociceptive stimuli in traumatized tissue [320]. In the same line, general anesthetics (GAs), which depress the CNS, can also activate peripheral nociceptive neurons. The pronociceptive effects of GAs combined with surgical tissue damage could even lead to a paradoxical increase in postoperative pain and inflammation [185]. Clinical concentrations of noxious intra-venal and inhalation GAs excite sensory neurons by selectively activating TRPA1. Isoflurane and desflurane are pungent, while sevoflurane and halothane are not, and TRPA1-dependent neurogenic inflammation is more severe in mice anesthetized with pungent- compared with nonpungent anesthetics.

A further surprise came with the identification of local anesthetics (LAs) as activators of the irritant receptor TRPA1. Lidocaine, inhibits cellular excitability by blocking voltage-gated Na⁺ channels, but can activate TRPA1 in a concentration-dependent manner. This activation is blocked by the TRPA1-antagonist HC-030031. Interestingly, lidocaine can also act as an inhibitor of TRPA1, an effect more evident with rodent than human TRPA1. This species-specific
difference is probably linked to the pore region (S5 and S6) [167]. Local anesthetics like lidocaine and procaine also mediate glutamatergic spontaneous excitatory transmission in substantia gelatiosa (SG) neurons. Lidocaine dose-dependently and reversibly increases the frequency but not the amplitude of spontaneous EPSCs in SG neurons. This presynaptic enhancement is due to activation of TRPA1 in nerve terminals presynaptic to SG neurons. Lidocaine dose-dependently increases glutamatergic spontaneous excitatory transmission in sub-

Fenamate nonsteroidal anti-inflammatory drugs (NSAIDs) can also activate and sensitize TRPA1. Several non-electrophilic NSAIDs, including flufenamic, niflumic, and mefenamic acid, as well as flurbiprofen, ketoprofen, diclofenac, and indomethacin, reversibly activate TRPA1. The response to fenamate agonists is blocked by TRPA1 antagonists. Fenamate NSAIDs also potentiate the activation of TRPA1 by electrophilic compounds [122].

Non-electrophilic compounds can also be metabolized to electrophilic products that can affect TRPA1 function. N-Acetyl-p-aminophenol (paracetamol, acetaminophen, APAP) is the most common antipyretic/analgesic medicine worldwide. In case of APAP overdose, its metabolite, N-acetyl-p-benzoquinoneimine (NAPQI), leads to important liver damage. NAPQI, like other TRPA1 activators, is an electrophilic molecule and stimulates TRPA1, causing airway neurogenic inflammation. This inflammatory responses evoked by NAPQI and APAP can be abolished by TRPA1 antagonists [204].

Very often compounds used in therapeutics and cosmetics are potent TRPA1 activators. Parabens, alkyl esters of p-hydroxybenzoate often added to pharmaceuticals, cosmetics and food products as antibacterial agents can behave as TRPA1 activators [95]. Calcium antagonists such as 4-different 1,4-dihydropyridines (nifedipine, nimodipine, nicardipine and nitrendipine) and the structurally related L-type calcium channel agonist BayK8644, exert powerful activating effects on TRPA1. Activation by nifedipine is reduced by camphor and the selective TRPA1 antagonist HC03001 [90]. Similarly, 5-nitro-2-(3-phenylpropylamino)-benzoate (NPPB), a classic C\textsuperscript{2}\textsuperscript{r} channel blocker, potently activates human TRPA1. The action of NPPB suggests a possible close interaction between S5 and the N-terminal domains of the channel. As indicated by the analysis of NPPB derivatives, NPPB activates TRPA1 through a structure-specific mechanism [171].

Primary alcohols cause skin, eye or nasal irritation via activation of TRPA1. Higher alcohols such as 1-butanol, 1-hexanol and higher, activate TRPA1 proportional to the carbon chain length, i.e. the potency increased with the carbon chain length. Interestingly, activation by primary alcohols also requires Cys665 as well as His983 residues in the N-terminal part of TRPA1 [151].

In general, TRPA1 is a non-covalent sensor of polyunsaturated fatty acids (PUFAs), which contain at least 18 carbon atoms and three unsaturated bonds. Those PUFAs activate TRPA1 to excite primary sensory neurons and endocrine cells. They act non-covalently and independently of known ligand binding domains located in the N-terminal [195].

Many non-covalent modulators of TRPA1 function in a bimodal fashion, i.e. they activate the channel at low concentration, and inhibit it at higher concentrations. Menthol from Mentha piperita, a known TRPM8 activator, is also a bimodal modulator of TRPA1. Low-micromolar concentrations of menthol cause channel activation, whereas higher concentrations lead to a reversible channel inactivation [134]. This is only true for human TRPA1 whereas its non-mammalian versions are insensitive to menthol. Mouse-human TRPA1 chimeras reveal that the pore region between S5 and S6 is the critical domain determining whether menthol can act as an inhibitor. Specific residues, Thr877, Ser876 and Gly878 are critical for menthol responsiveness but determine also the function of other nonreactive TRPA1 modulators, such as binding of the blocker AMG 5445 and AP18 [305]. Similarly to menthol, the super-cooling agent icilin (AG-3-5) activates not only TRPM8 as but also TRPA1. Icilin can induce a rapid, long-lasting and dose-related hyperthermia in rats. Pretreatment with NOS inhibitor N(G)-nitro-L-arginine methyl ester hydrochloride (L-NAME) attenuate this hyperthermia. TRPM8/TRPA1 activation induced hyperthermia requires both NO production and N-methyl-D-aspartate (NMDA) receptor activation [70].

Another example of species-dependent activator of TRPA1 is caffeine. Caffeine from Coffea arabica, activates mouse TRPA1 but suppresses its human version. The region between residues Thr231 and Asp287 in the distal N-terminal cytoplasmic region of mouse TRPA1 is critical. The mutation of mouse TRPA1, Met268Pro, changes the effect of caffeine from activation to suppression [198, 199]. Similarly, nicotine from Nicotinia tabacum or its analogue, anabasin from Nicotiana glauca, are bimodal TRPA1 modulators. Topical application of nicotine, as used in nicotine replacement therapies, causes irritation of the mucosa and skin due to TRPA1 activation. In contrast, higher concentrations inhibit the channel. It is thought that blocking of TRPA1 activation may facilitate the development of smoking cessation therapies with less adverse effects [273].

The gating promiscuity of TRPA1 is also illustrated by the effects of several metals. Zinc, an essential biological trace element, is required for the structure or function of over 300 proteins. High concentrations of zinc have cytotoxic effects and can cause pain and inflammation. Surprisingly, zinc activates TRPA1 through a unique mechanism that requires zinc influx through TRPA1 channels and subsequent activation via specific intracellular cysteine and histidine residues. TRPA1 is highly sensitive to intracellular zinc, as low nanomolar concentrations activate TRPA1 and modulate its sensitivity [121].
antioxidants and antimalarial drug clioquinol (CQ) was withdrawn from the market when it was linked to an epidemic of subacute myelo-optico-neuropathy (SMON). Clioquinol exerts its anti-parasitic actions by acting as a Cu/Zn chelator and ionophore. Local injections of CQ produce mechanical hyperalgesia and cold hypersensitivity through activation of TRPA1 in a Zn\(^{2+}\)-dependent manner. Direct application of Zn\(^{2+}\) to the intracellular face of excised, inside-out patches activates TRPA1. Thus, TRPA1 acts as a sensor of intracellular Zn\(^{2+}\), and Zn\(^{2+}\) ionophores, such as CQ, activate TRPA1 by increasing intracellular Zn\(^{2+}\) concentrations [4]. Similarly to zinc, two other heavy metals, cadmium and copper also directly activate TRPA1, resulting in stimulation of pulmonary sensory neurons [110].

In the light of the increasing use of non-pungent capsaicinoids as slimming agents to enhance energy metabolism by activating sympathetic nervous, it is interesting to highlight that TRPA1 is also activated by these compounds, e.g. capsiate. Capsainoids activate TRPA1 by an as yet unknown mechanism, which does not involve covalent modifications of reactive Cys or Lys residues [253]. Capsainoids are unstable compounds that quickly generate electrophilic quinone methides, and this mechanism, somewhat similar to that involved in the activation by the metabolites of APAP, might underlie their action on TRPA1 [172].

Last but not least, TRPA1 is also activated by Δ9THC, the psychoactive compound in marijuana [129, 219, 260]. Also two non-psychoactive cannabinoids, cannabidiol (CBD) and cannabichromene (CBC), are known to modulate TRPA1. Specific agonists of TRPA1 channels and synthetic inhibitors of endocannabinoid cellular reuptake exert effects similar to those of CBC and CBD, stimulating descending pathways of anti-nociception and causing analgesia by interacting with several target proteins involved in nociceptive control [64, 179]. THC-induced CGRP release and vasorelaxant responses to sensory nerve stimulation also proceed via TRPA1 activation [301]. Morphine and its derivatives and analogues are key drugs in pain control but are proalgesic at high concentrations, and short-lasting painful sensations are known upon dermal application of morphine. Just like cannabinoids, morphine as well as naloxone induce release of CGRP via activation of TRPA1 [93]. Interestingly, TRPA1 is also activated by CB antagonists, e.g. AM251 and AM630, representing an alternative target for these drugs [222]. Six phytocannabinoids [CBD, THC, CBD-acid, THC-acid, cannabichromene (CBC) and cannabigerol (CBG)] showed potent TRPA1 activating properties. None of these compounds could activate TRPM8 but, with the exception of CBC, they could all antagonize TRPA1 activation by menthol or icilin. The unusual cannabinoxid sesqui-CBG, isolated from the waxy fraction of a variety of fiber hemp (Cannabis sativa) could inhibit TRPA1 responses more potently than its lower prenologue CBG [228], suggesting that, by proper chemical manipulation, the TRPA1 activating and inhibiting properties of cannabinoids could be dissected. Clearly, phytocannabinoids and cannabis extracts exert some of their pharmacological actions by interacting with TRPA1 and possibly TRPM8 channels, with potential implications for the treatment of pain and cancer [65].

TRPA1 antagonism

The huge importance of TRPA1 in pain, inflammation and many other potential indications in acquired diseases has initiated an increasing demand for specific antagonists. The first TRPA1 antagonists were reported in 2007, and were based on a xanthine structure, as exemplified by HC-030031 (Hydrea company), still the most widely used product. More recently, phthalimide derivatives (e.g. the high nano-molar ligand Glenmark 17, 34) and imidazo-uridine derivatives (e.g. Glenmark 8, 39) were disclosed by the Glenmark company. All these inhibitors are non-electrophilic, and presumably act via non-covalent interaction. Interestingly, also camphor, a monoterpine derived from Cinnamomum camphora, acts as a natural TRPA1 antagonist [8, 16, 236, 246].

Among electrophilic compounds, several oximes have been recently disclosed as antagonists of TRPA1. Oximes related to AP18 possess both agonist and antagonist activity [66]. Another oxime-derived compound, A-967079, is a potent nano-molar blocker of human TRPA1 that attenuates cold allodynia produced by nerve injury but does not alter noxious cold sensation and does not alter body temperature [51]. Other electrophilic agonist with a nano-molar range of action are Abbott A, Renovis 11, Amgen AMG 7160, 2504, 9090, 5445, and the Abbott CMP1, 2, 3 [236]. Recently, a new series of 7-substituted-1,3-dimethyl-1,5-dihydropyrido[3,2-d]-pyrimidine-2,4-dione derivatives have been developed as efficient TRPA1 antagonists [21].

Interestingly, heat suppresses the activation of TRPA1. Thus, agonist-induced TRPA1 currents in HEK293 cells are suppressed by warm temperatures, and almost abolished at 39 °C. This inhibition occurs when TRPA1 is activated by both electrophilic and non-electrophilic agonists. Warming also attenuates TRPA1 mediated ionic currents in sensory neurons, which may explain, the well-know effect of warmth to attenuate pain [293].

Activation by cold

TRPA1 has been initially described as a cold-activated channel [14, 260], but this view has now been questioned ([46, 129], for a critical review see [163]). TRPA1-expressing primary afferents mediate noxious cold responses in anesthetised rats, but the TRPA1 agonist cinnamaldehyde applied to the skin in anesthetised rats did not sensitize noxious cold-
evoked hind limb withdrawal. However, TRPA1 agonist sensitized the noxious reflex withdrawal to heat, but not cold. Block of the TRPA1 did not inhibit cold evoked activity in either cinnamaldehyde sensitive or insensitive cold responsive nociceptors. These results do not support the hypothesis that TRPA1-expressing cutaneous afferents play an important role in noxious cold responses [78].

However, recent papers have provided fairly convincing evidence for direct activation of TRPA1 by cold, and have identified a central role in pain for this most “sensitive” sensory channel [159]. Thus, TRPA1 is activated by cold in a Ca²⁺-independent and Ca²⁺ store-independent manner [137, 246]. Cold plate and tail-flick experiments have also revealed a TRPA1-dependent, cold-induced nociceptive behavior in mice [68, 137], and it has been clearly demonstrated that TRPA1-deficient mice display the loss of a specific subset of cold-sensitive TG neurons. Similarly, TRPA1 also contributes to cold-induced contractions in isolated rat colon preparations [74]. Application of cold temperature to these visceral afferents can evoke major protective reflexes and thermoregulatory responses, and TRPA1 is the major mediator of cold-evoked responses in vagal visceral neurons. Most cold-evoked responses are potentiated by TRPA1 agonists such as cinnamaldehyde, menthol, and icilin, whereas camphor and HC03001 act as blockers [89].

Mechano-activation

The mechanosensory role of TRPA1 and its contribution to mechanical hypersensitivity in sensory neurons still remains enigmatic. As an indication of mechanosensitivity, hypertonic solution (HTS), but not hypotonic solutions, activates TRPA1. Single-channel recordings show no difference in conductances as compared with electrophilic activation. In rat DRG neurons, HTS-evoked TRPA1 currents are blocked by camphor. HTS depolarizes the membrane potential of DRG neurons leading to the generation of action potentials [319]. Another indication comes from the use of amphipathic molecules such as trinitrophenol (TNP) and chlorpromazine (CPZ). Trinitrophenol causes a membrane curvature that leads to activation of TRPA1 [117]. In contrast, CPZ blocks TRPA1 at positive potentials and activates at negative potentials [117].

Several evidences from in vivo experiments support a role for TRPA1 as a mechanosensor. TRPA1 at sensory afferent terminals in skin is required for their responsiveness to both noxious chemical and mechanical stimuli. In skin-nerve preparations, acute application of the selective TRPA1 antagonist HC-030031 inhibits all formalin responses in rat C fibers. HC-030031 could also markedly reduce the mechanically-evoked action potential firing in rat and wild type mouse C fibers, particularly at high-intensity forces, but has no effect on the mechanical responsiveness of Aδ fiber nociceptors. In TRPA1-deficient mice, HC-030031 has no effect on mechanically-evoked firing, indicating a TRPA1-dependent mechanism [138]. TRPA1 is detected not only on many thin-caliber axons and intraepidermal endings, but also on many large-caliber axons as well as lanceolate and Meissner endings. Epidermal and hair follicle keratinocytes also express TRPA1. Thus, in nociceptor terminals TRPA1 modulates mechanotransduction via a cell-autonomous mechanism that very likely requires its modulatory role in keratinocytes that may interact with sensory terminals to modify their mechanical firing properties [160]. Rapid focal mechanical stimulations evoke mechanically-sensitive currents in DRG neurons. Small neurons (diameter < 27 μm) expressing TRPA1 give rise to C-fiber afferents in vivo and show immediately- (IAMC), rapidly- (RAMC) or slowly-adapting (SAMC) mechanically-activated currents. TRPA1 deletion in knockout mice significantly reduced maximum IAMC whereas other current types were unaltered in small- and large-diameter neurons. The HC-030031 antagonist significantly decreased IAMC amplitudes in TRPA1 wild type neurons. TRPA1 makes a specific contribution to normal mechanosensation in a distinct subset of DRG neurons and has the capacity to tune neuronal mechanosensitivity depending on its degree of activation or expression [35, 285].

TRPA1 might also function as a mechanosensor under pathophysiological conditions. Inflammatory hypersensitivity is characterized by behavioral reductions in withdrawal thresholds to noxious stimuli. Skin primary afferents have lowered thermal thresholds in inflammation, but whether these mechanical thresholds are altered remains controversial. Interestingly, a subset of afferents that is sensitized to mechanical stimulation by inflammation expresses TRPA1 [77]. Similarly, TRPA1 is implicated in inflammatory pain in the gastrointestinal (GI) tract. Sensory information from the GI tract is conducted via different afferent fibers and different pathways. Nodose ganglia and DRGs whose neurons innervate 3 different regions of the GI tract, all express TRPA1. TRPA1 is required for normal mechano- and chemosensory function in specific subsets of vagal, splanchnic and pelvic afferents. Obviously, TRPA1 has mechanosensory function and mediates noiception within the viscera in normal and inflamed tissue [36]. Very likely TRPA1 alone is unable to mediate responses to mechanical stimuli, but might be involved in the formation of mechanosensory complexes with other yet to be identified sensory proteins.

Ca²⁺ dependent modulation

Ca²⁺ is one of the most important endogenous modulators of TRPA1. It mediates both potentiating (activating) and
inactivating (desensitizing) effects that are still not fully understood. TRPA1 is affected by \([Ca^{2+}]_c\), that can be entirely attributed to entry through TRPA1 and subsequent elevation of intracellular calcium. The mutation of Asp918 in the putative TRPA1 pore greatly reduces \(Ca^{2+}\) permeability. Extracellular \(Ca^{2+}\) alone produced neither potentiation nor inactivation. Both processes were restored by reducing intracellular \(Ca^{2+}\) buffering. Application of \(Ca^{2+}\) to the cytosolic face of excised patches is sufficient to produce both potentiation and inactivation of TRPA1 channels. Moreover, in whole cell recordings, elevation of intracellular \(Ca^{2+}\) potentiated, but did not inactivate TRPA1. Thus, potentiation and inactivation are two independent processes. TRPA1 currents could be inactivated by \(Mg^{2+}\), \(Ba^{2+}\) and \(Ca^{2+}\), but potentiating effects were only observed for \(Ba^{2+}\) and \(Ca^{2+}\) [289].

Phosphatidylinositol-(4, 5)-bisphosphate-dependent modulation

Most if not all TRP channels are regulated by phosphatidylinositol-(4, 5)-bisphosphate (PI(4, 5)P\(_2\)) that causes channel activation or sensitization, while depletion of PI(4, 5)P\(_2\), e.g. via PLC-dependent mechanisms, renders TRP channels inactive [209]. The situation for TRPA1 is not completely clear. A typical feature of TRPA1 is its rapid desensitization following activation by agonists such as MO, cinnamaldehyde, and a high intracellular \(Ca^{2+}\) concentration. TRPA1 desensitization is delayed when PI(4, 5)P\(_2\) is supplied via the patch pipette whereas neomycin, a PI(4, 5)P\(_2\) scavenger, accelerates desensitization. Preincubation with the phosphatidylinositol-4-phosphate-5-kinase (PI-4 kinase) inhibitor wortmannin reduces both constitutive TRPA1 channel activity and the response to MO. This set of data confirm that PI(4, 5)P\(_2\) modulates TRPA1, albeit to a lesser extent than other known PI(4, 5)P\(_2\)-sensitive TRP channels, such as TRPM4 [135]. Other reports show that in inside-out patches, PI(4, 5)P\(_2\) does not activate TRPA1. When TRPA1 is activated by electrophilic agonists, addition of PI(4, 5)P\(_2\) produced a concentration-dependent inhibition of TRPA1. Apparently, PI(4, 5)P\(_2\) may act as an inhibitor of TRPA1, reducing its sensitivity to activators [140].

This view is further complicated by a possible TRPV1/TRPA1 interaction. In DRG neurons, hydrolysis and accumulation of PI(4, 5)P\(_2\) modulate both TRPV1 and TRPA1 activities. Inflammation results in a long-lasting PI(4, 5)P\(_2\) depletion. A chronic PI(4, 5)P\(_2\) production can be stimulated by overexpression of PI-4 kinase, while the PI(4, 5)P\(_2\) -specific phospholipid 5’-phosphatase can reduce plasma membrane levels of PI(4, 5)P\(_2\). However, agonist responses of TRPA1 are not significantly influenced by chronic changes in PI(4, 5)P\(_2\). However, when TRPA1 and TRPV1 are present, chronic PI(4, 5)P\(_2\) reduction leads to pronounced tachyphylaxis of both channels. Thus, chronic effect of PI(4, 5)P\(_2\) on TRPA1 activity depends on presence of the TRPV1 [220], and the possible formation of mixed TRPV1-TRPA1 oligomers whose regulation differs from that of the single channels.

Modulation by phosphorylation and polyphosphates

TRPA1 is likely to be modulated by phosphorylation. It is known that TRPA1 can be activated by bradykinin [23]. This process involves activation of PKA, that depends on cyclic AMP (cAMP) and probably induces channel phosphorylation and sensitization [8, 291].

One difficulty to study effects of phosphoinositides in detail, like PI(4, 5)P\(_2\), is represented by the need to take long recordings in inside-out configurations. Probably, the function of TRPA1 requires an unidentified cytosolic factor whose action can be mimicked by inorganic polyphosphates. Polyphosphates (e.g. pyrophosphate (PPI), polytriphosphates (PPII)) are intracellular molecules that are known to be able to rescue activation of TRPA1 by covalent modification in inside-out patches [139]. It has been postulated that TRPA1 can switch from a conformation insensitive to pungent chemicals to a sensitive one, or, alternatively, that TRPA1 becomes completely non-functional and insensitive to all activators when the cytosolic factor is absent. In this scenario, polyphosphates could stabilize the inside-out patch configuration, a view supported by the observation that \(Ca^{2+}\) fails to activate TRPA1 in inside-out patches unless polyphosphates are present. Therefore, TRPA1 might exist in different functional states: a native state (cell-attached patch) and a non-native state (excised patch). Interestingly, THC can activate TRPA1 even in the absence of polyphosphates, whereas electrophilic pungent chemicals and \(Ca^{2+}\) require polyphosphates for activation [49].

Modulation by protein—protein interaction

Interactions of TRP channels with diverse modulatory proteins or even \(\beta\)-subunits are an increasingly important research topic. So far, the best studied TRPA1 partners are TRPV1, the ubiquitin hydrolase CYCL, the PKA anchor protein AKAP5 and secretogranin 3, a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins that serve as precursors for biologically active peptides (see also http://trpcchannel.org/).

Some features of neuronal TRPA1 are not present in heterologous expression systems but can be restored when TRPA1 and TRPV1 channels are co-expressed [243]. TRPV1 and TRPA1 function together and resiniferatoxin-mediated “neurosurgery” removes both sensor elements. In adult mice, resiniferatoxin causes desensitization to heat and sensitization to cold, resulting in cold hyperalgesia [223]. \(Ca^{2+}\)-triggered
activation of TRPA1 is attenuated by the presence of TRPV1 in the presence of extracellular Ca²⁺ but not in Ca²⁺-free conditions. TRPV1 mutations at Tyr671 probably affect TRPA1 permeation properties, but the mutations in TRPV1 do not affect association of TRPA1 and TRPV1 channels. The mutation of Tyr671 to lysine in TRPV1 altered the magnitude of currents through TRPA1, the sensitivity of [Ca²⁺]e and the mutation of Tyr671 to lysine in TRPV1 altered the magnitude of currents through TRPA1, the sensitivity of [Ca²⁺]e and the voltage-dependency [221]. The synthetic cannabinoid, arachidononyl-2 chloroethanolamine (ACEA) activates TRPV1, but inhibits TRPA1 probably via a TRPV1-dependent mechanism. Some cannabinoids mediate their peripheral analgesic properties, at least in part, via the TRPV1 and TRPA1 channels [240].

Analysis of icilin effects on TRPA1 and sensitivity of mice to cold stimuli, which is inhibited by blockers (BEL, bromoenol lactone) of the sub-type of phospholipase A2, iPLA2, has suggested a possible interaction of TRPA1 with this lipase [102]. The mammalian prokineticins PK1 and PK2, and their G-protein coupled receptors prokineticin receptor 1 (PKR1) and prokineticin receptor 2 (PKR2) have been identified and linked to several biological effects as gut motility, neurogenesis, angiogenesis, circadian rhythms, hematopoiesis, and nociception. Mice lacking PKR genes exhibit impaired PK1, PK2 mediated hyperalgesia, which partially depend on TRPA1. Both receptors may interact with TRPA1 [207]. Another link between pain and TRPA1 comes from studies on the protease activated receptor 2, PAR2. TRPA1 is functionally coupled with PAR2 and both proteins are co-localized in DRG. PAR2 activation potentiates TRPA1 activation probably via PLC. Application of phospholipase C (PLC) inhibitors or phosphatidylinositol-4,5-bisphosphate PI(4, 5)P₂ suppressed this potentiation indicating that PI(4, 5)P₂ might be a TRPA1 inhibitor rather than an activator. This effect is probably mediated via a coupling between TRPA1 and PAR2 [63, 314].

A dietary link to TRPA1 modulation

A huge number of TRPA1 modulators are naturally produced in plants, fungi and animals to induce avoidance behavior in predators or competitors. The best known examples are the many culinary plants that produce structurally unrelated pungent or irritating compounds.

Allium sativum (garlic) has the enzymatic machinery to generate the pungent electrophilic allicin [174], an organosulfur compound that acts as a precursor for a host of compound that includes diallyl sulphide, diallyl disulfide and diallyl trisulfide. In a similar way, allyl isothiocyanates can be generated from wasabi, horse radish and mustard oil. These compounds are pungent and cause inflammations, edemas and hyperalgesia [129]. Garlic, and particularly allicin, protect against the development of right ventricular hypertrophy and reduces right ventricular pressure [263]. This protective effect is due to an action on vascular endothelium preventing endothelial cell dysfunction. Boiled or aged garlic, which cannot generate allicin from its amino-acidic precursor due to enzymatic denaturation, has no protective effect and is non-pungent. Since TRPA1 is expressed in heart, it is intriguing to speculate that this channel might be contributing to these beneficial effects [259].

The spice wasabi (from Wasabia japonica) acquires pungency not only by AITC, but also by related isothiocyanates [6-(methylsulfinyl)hexyl isothiocyanate (6-MSTIC) and 6-(methylthio)hexyl isothiocyanate (6-MTITC)], that are less pungent but contribute to the fresh flavor of wasabi. All these isothiocyanates activate TRPA1 in an electrophilic manner [282].

Another interesting compound from garlic, ajoene, is an unsaturated compound that contains a reactive thiophilic disulfide bond, and that acts as antithrombotic (anti-clotting) agent. Ajoene cannot directly activate TRPA1, but it can enhance activation of TRPA1 by electrophiles, potentiating the depolarization that they induce [309].

The drimane sesquiterpenes isovelleral and polygodial are noxious and pungent terpenes characterized by reactive α,β-unsaturated 1,4-dialdehyde moiety. These small molecules of fungal and animal origin are all TRPA1 agonists [88],and are the archtypical members of a large class of dialdehydes that also includes miogadial (MD), miogatrial (MT), and polygodial (PG), and that show equal or even stronger TRPA1 agonistic activity than AITC from MO [126]. Miogadial, isolated from flower buds of the myoga ginger (Zingiber mioga) or from water pepper (Polygonum hydropiper) has a pleiotropic biological profile that includes antimicrobial activity against Gram-positive bacteria and yeasts as well as chemopreventive potentials. Leaves of water pepper contain polygodial that elicits a warm and pungent flavour. Polygodial has strong antifungal and antimicrobial activities and acts as anti-hyperalgesic, anti-nociceptive, anti-inflammatory, anti-allergic agent and vasorelaxant agent [10]. The related sesquieterpenes isovelleral, isolated from the fungus Lactarius vellereus, render it inedible because of its peppery taste. Isovelleral contains a β-unsaturated dicarbonyl moiety potentially capable of forming Michael adducts. Interestingly, it can also activate TRPA1 with mutations of reactive cysteines. Thus, dialdehyde sesquiterpenes may activate TRPA1 through a mechanism that differs from other electrophiles [20]. Interestingly, some of these compounds are also activators of TRPV1 (see for a review [286]).

The main pungent component in galangal (Alpinia galangal) is 1’-acetoxychavicol acetate (ACA). ACA does not interact with TRPV1, but activates TRPA1 with a potency higher than MO [201]. Galangal has an interesting ethnopharmacology that includes stimulation of the appetite, promotion of the blood circulation in the brain and strengthening...
of sexuality. When consumed in large amounts, it can also generate visual hallucinations.

Cinnamaldehyde from *Cinnamomum verum* is the main constituent of cinnamon oil. It is widely used as a spice in cookery as a condiment and flavoring material in chocolate and many dessert recipes, such as apple pie, donuts, and spicy candies. Interestingly, oral application of cinnamaldehyde causes burning and tingling due to activation of TRPA1 [14, 20].

Szechuan peppers from various *Zanthoxylum* species and the Japanese pepper tree (*Z. Piperitum*, sansho) contain a mixture of alkaloids that includes hydroxy-α-sanshool, a compound that causes irritations, cooling, tingling and sometimes paresthetic sensations on the tongue and strongly stimulates salivation [189]. Hydroxy-α-sanshool but not hydroxy-β-sanshool depolarizes sensory neurons with concomitant firing of action potentials and evokes inward currents due to activation of TRPA1 and TRPV1 [153]. Additionally, hydroxy-α-sanshool activates a subset of sensory DRG neurons by inhibiting 2-pore potassium channels, KCNK3, KCNK9, KCNK18 [25]. A tingle-evoking sanshool analog, isobutylalkenyl amide (IBA), excites rat DRG neurons and causes unfamiliar aversive sensations probably mediated by activation of TRPM8, TRPA1 and TRPV1 [146]. Interestingly, Szechuan pepper also contains an abundant acyclic terpene alcohol, linalool, which also activates TRPA1. Linalool, as well as 6-shogaol and 6-paradol from ginger (*Zingiber officinale*), act on TRPA1 by covalent bonding whereas none of these compounds activated TRPV1 with this mechanism [237].

Curcumin, the active principle of turmeric root (*Curcuma longa*), is used in many cuisines as “yellow curry”. It provides pain relief, probably by TRPA1 induced slow depolarization followed by Na+ channel inhibition in sensory ganglia and/or fast desensitization of the channel after activation [164]. Numerous clinical trials in humans are underway, studying the effect of curcumin on various diseases, including multiple myeloma, pancreatic cancer, myelodysplastic syndromes, colon cancer, psoriasis, and Alzheimer’s disease and the compound might have interesting application in oncology, both as a selective cytotoxic agent, and in cancer supportive care [114].

*Perilla frutescens* from the Lamiaceae family is a food plant widely used in Asian cuisine known for its interesting taste also called beefsteak plant, Chinese basil, purple mint or rattlesnake weed. The red perilla is used mostly in fish stews in China whereas the green one is more commonly found in Korean and Japanese cuisines as pickled green perilla with red chili powder and soy sauce. The active compounds in perilla, perillaldehyde and perillaketone, are both activators of TRPA1. Perilla leaves (zisuye) are widely used in traditional Chinese medicine as a remedy against stomach dysfunction [22].

From the berries of black pepper (*Piper nigrum*) 19 compounds that are TRPV1 and TRPA1 activators have been characterized. Dried pepper corn has been used since antiquity for both its flavour and as a medicine. Piperine (causing the spiciness of black pepper), isopiperine, isochavicine, piperonine, pipereol, piperolein B, and N-isobutyl-(2E,4E)-tetradeca-2,4-diamide also strongly activate both TRPA1 and TRPV1 [216].

The thyme plant (*Thymbra vulgaris*) contains thymol (also known as 2-isopropyl-5-methylphenol), is a natural monoterpenic phenol derivative of cymene isomeric with carvacrol. Thymol has a pleasant aromatic odor with a relatively pungent flavor, and is often used to flavour meats, soups and stews. Thymol is a member of natural compounds known as biocides that have strong antimicrobial and antifungal properties. Thymol, as well as closely related carvacrol derived from *Origanum vulgare*, can reduce bacterial resistance to antibiotics. Additionally, there is growing evidence that thymol has antitumor properties. Thymol directly activates TRPA1 and sensitizes it to further exposure to thymol and carvacrol [165, 306]. The response to thymol is blocked by camphor [165]. Thymol-related phenols such as 2-tert-butyl-5-methylphenol and 2,6-disopropylphenol (propofol) also activate TRPA1 [306].

*Salvia officinalis* is used as a traditional herbal medicine for gastric disturbances and inflammatory processes. Hydro-alcoholic extract (HE) from salvia leaves, containing carnosol and ursolic acid/oleanolic acid, presents significant anti-inflammatory and also antinociceptive effects very likely through modulation of TRPA1 [238].

The California bay laurel *Umbellularia californica* can be used in cuisine as a replacement of laurel (*Laurus nobilis* L.), but has also been nicknamed ‘the headache tree’ because inhalation of its vapours can cause severe headache crises. The mechanism of the headache precipitating properties of *Umbellularia californica* has recently been clarified and traced to the presence of elevated concentrations of the monoterpenic ketone umbellulone, a reactive compound that rapidly binds thiols, shows irritating properties, and can stimulate TRPA1 [205, 322].

Ligustilide, the major aroma constituent of celery (*Apium graveolens*), is an electrophilic volatile dihydroththalide that activates TRPA1 but is also capable of inducing a modest block of activated TRPA1. The action of ligustile on TRPA1 contributes to the gustatory effects of celery, its major dietary source, and to the pharmacological action of popular plants used in traditional Chinese (*Angelica sinensis, Ligusticum chuanxion*) and native American (*Ligusticum portiere*) medicine [323].

A characteristic astringent taste is the gustatory hallmark of polyphenols and tannins. Catechins and their polymers are the most abundant polyphenols in wine and tea. A typical green tea polyphenol is epigallocatechin gallate
(EGCG). EGCG activates TRPA1 and might play an important role in the astringency taste on the tongue [158]. Oleocanthal, an anti-inflammatory compound contained in extra-virgin olive oil, elicits an unusual oral pungency sensed almost exclusively in the throat. This rare irritation pattern is a consequence of both the specificity of oleocanthal for a single sensory receptor, and the anatomical restriction of this sensory receptor to the pharynx, within the oral cavity. Oleocanthal selectively activates TRPA1 but not TRPV1. The over-the-counter analgesic, ibuprofen, which elicits the same restricted pharyngeal irritation as oleocanthal, also specifically excites rodent sensory neurons via TRPA1 through a mechanism that is different from the covalent cysteine modification [226].

Eugenol from cloves (Syzygium aromaticum) is a phenylpropene used in perfumeries, flavorings (very strong taste), and medicine as a local antiseptic and anesthetic. Similar properties are shown by gingerols, a class of pungent compounds from fresh ginger (Zingiber officinale). Both eugenol and gingerol activate TRPA1 channel [20]. Interestingly, some analogues of gingerols are potent TRPA1 antagonists [194].

Resolvins are synthesized in the human body from a fish oil derived omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Resolvin D1 (RvD1), a naturally occurring anti-inflammatory and pro-resolving lipid molecule inhibits TRPA1 at nanomolar and micromolar levels. Consistent attenuations can be observed in agonist-specific acute pain behaviours. RvD1 is a novel endogenous inhibitor for several sensory TRPs such as TRPA1, TRPV3 and TRPV4 [17].

Other TRPA1-active natural compounds such as caffeine, nicotine, menthol and camphor had already been discussed in previous sections.

Physiological functions of TRPA1

Sensory functions

As already described in the previous sections, TRPA1 mainly acts as a chemosensor involved in nociception. Interestingly, during development, TRPA1, similarly to other TRP channels, emerges in waves of differentiation. This process starts at embryonic day 12.5 (E12.5) and extends into the postnatal life. Most likely, TRPA1-expressing sensory neurons derive from the population of TRPV1-expressing neurons, and form two distinct classes of nociceptors: around birth in the peptidergic population, and after P14 in the nonpeptidergic class [120].

TRPA1 is a typical sensor of cell threat. Sensing of TRPA1 activating compounds can provide warning of potential damage rather than actual damage itself. Many TRPA1 activators are also lachrymators in the eye, which would thus sense toxic and irritant compounds, such as α-terpineol, ambroxol, benzaldehyde, and toluene, before they reach the upper airways [28–30].

TRPA1 is necessary for development of inflammatory hypersensitivity and its activity is functionally modulated by growth factors that regulates sensitivity of sensory neurons such as the nerve growth factor (NGF), the glial cell line-derived neurotrophic factor (GDNF) and artemin, a neuronal survival and differentiation factor. Short-term application of artemin inhibits TRPA1 and plays a role in the regulation of sensory neurons for nociception [313]. However, overexpression of artemin enhances expression of TRPA1 in sensory neurons. This situation occurs in chronic inflammation and causes an increased sensitivity to noxious cold [86, 180].

Thermoregulation

TRPA1 agonists enhance thermogenesis and inhibit heat diffusion [182]. Icilin, a TRPM8/TRPA1 agonist, produces a dose-related hyperthermia in rats which requires both NO production and NMDA receptor activation. However, it is difficult to differentiate between the role of TRPM8 and TRPA1 [70]. TRPA1 activation alone also causes hyperthermia [235]. Different to TRPV1 inhibitors, the selective blockade of TRPA1 channel attenuates pathological pain without altering noxious cold sensation or causing hypothermia [51]. Thus, TRPA1 activation seems to have a mild effect on global thermoregulation.

Cell barrier function

TRPA1-expressing vagal sensory afferents innervating airways and abdominal tissues mediate axonal chemosensitivity and control of neuropeptide release. The activation of TRPA1 evokes CGRP release and becomes important during increasing vascular permeability in inflammation or neuropathy [299].

Gastro—intestinal system

TRPA1 plays a role in the gastric accommodation (or gastric relaxation) response that reduces meal-induced increase in gastric pressure and thus might impair the development of dyspeptic symptoms. Pretreatment with TRPA1 agonist increases the gastric tone via a TRPA1-mediated cholinergic neuronal pathway and subsequently inhibits meal-induced gastric accommodation [154]. The effect of satiety might also be partially related to activation of TRPA1. Selective TRPA1 agonists from spices may act within cranial visceral afferent pathways mediating satiety and contribute to reduction of the food intake associated with spicy diets [55].
TRPA1 also has an influence on GI motility. Secretory effects are induced by TRPA1 agonists. Mucosal application of agonists also induced CI and HCO3 secretion in a concentration-dependent manner whereas the serosal application has no effect. TRPA1 activation induces anion secretion through PG synthesis, independently of neural pathways in the colon, and is mediated mainly by the PG receptor EP4 subtype. Activation of TRPA1 in colonic epithelial cells is also involved in the host defense mechanism through rapid anion secretion [131].

Serotonin is abundantly present throughout the GI and is stored mostly in enterochromaffin (EC) cells located on the mucosal surface. Secretion of serotonin from EC cells stimulates both intrinsic and extrinsic nerves, which results in various physiological and pathophysiological responses, such as GI contractions. TRPA1 is highly expressed in EC cells. TRPA1 agonists stimulate EC cell functions by an increased serotonin release, which subsequently promotes ileum contraction via the serotonin receptor. Thus, TRPA1 acts as a sensor molecule for EC cells and regulates GI function [212].

**Cardiovascular system**

As TRPA1 is expressed in most endothelial cells and in arterial vessels, it is involved in regulation of the blood flow. It is probably localized and functional at the myoendothelial interface [233]. Activation of TRPA1 mediates endothelium-dependent smooth muscle cell hyperpolarization and vasodilation that requires the activity of small and intermediate conductance Ca2+-activated K+ channels. TRPA1 agonists cause transient depressor responses followed by sustained increases in heart rate and blood pressure that may result from elevated sympathetic nervous activity. These findings indicate that TRPA1 activity influences vascular function [79]. Gaseous transmitters such as hydrogen sulphide causes vasodilation probably via release of CGRP, a mechanism similar as that described for TRPV1 [300].

**Pancreas**

As described above, TRPA1 is expressed in pancreatic β-cells. Activation of TRPA1 by AITC, H2O2, 4-hydroxynonenal (4-HNE), and cyclopentenone prostaglandins (PGJ2) and a novel agonist methylglyoxal (MG) induces membrane currents, depolarization, and Ca2+ influx leading to generation of action potentials. Endogenous and exogenous ligands of TRPA1 cause Ca2+ influx and induce basal insulin release in pancreatic β-cells. TRPA1-mediated depolarization acts synergistically with KATP channel blockade to facilitate this process [45]. In addition, the increase in intracellular Ca2+ concentration seems to be due to an influx through the L-type voltage-dependent Ca2+ channels activated upon depolarization induced by electrophilic activation of TRPA1. Thus, TRPA1 appears to be functionally coupled with the L-type voltage-dependent Ca2+ channel and ultimately mediate insulin secretion [213].

**Bladder**

TRPA1 is present on unmyelinated nerve fibers within the urothelium, suburothelial space, and muscle layer as well as around blood vessels throughout the bladder. TRPA1 agonists increased micturition frequency and reduce the voiding volume. Similar changes in urodynamic parameters are also observed after disruption of the urothelial barrier with protamine sulfate. Intravesical TRPA1 activators initiate detrusor overactivity, indicating that TRPA1 has a role in sensory transduction in this organ.

Acrolein, which can be formed endogenously under conditions of inflammation, and is also a toxic metabolite of cyclophosphamide, are known activators of TRPA1 that induce cystitis in the bladder. Similarly, H2S is a TRPA1 activator potentially involved in inflammatory bladder disease [261]. Other examples are fatty-acid derived nitroalkenes that react with nucleophiles such as cysteine and histidine residues in a variety of susceptible proteins, including TRPA1. They activate TRPA1 on afferent nerve terminals in the urinary bladder and thereby increase bladder activity [11]. Thus, pathologic activation of TRPA1 may play a role in inflammation of the bladder. Future studies should therefore address a potential regulation of bladder TRPA1 in cystitis and the relative potential of TRPA1 versus TRPV1 ligands to treat cystitis-associated signs and inflammation symptoms [190, 261].

Importantly, patients with lesions in the central nervous system exhibit a voiding reflex after perfusion of the bladder with cold solutions (ice water), which is absent in healthy volunteers. The role of TRPA1 in this phenomenon needs to be urgently evaluated because of its importance to differentiate peripheral from central bladder dysfunction. Patients without neurological diseases have a heightened cold bladder perception during the ice water test than patients with neurological diseases [5, 67].

**TRPA1 in other animal models**

In *C. elegans*, genetic approaches show that mutations in trpa-1 cause specific defects in mechanosensory behaviors related to nose-touch responses and foraging [145]. In addition, PVD neurons, multidentritic nociceptors in the body wall of *C. elegans* with long undifferentiated processes, express TRPA1 that confers response to harsh touch and also to cold temperatures [50].

The fruit fly expresses multiple TRPA channels. Drosophila TRPA1s respond to reactive electrophilic compounds
leading to nociception (avoidance). The nociceptive function of TRPA1s requires their expression in the nervous system, specifically within nociceptive multi-dendritic (MD) sensory neurons [206]. The Drosophila TRPA family member, painless, is expressed in a subset of MD neurons in the larval epidermis. This TRPA-type channel is required for larval heat and mechanical nociception. Mutations of painless also results in a defect in male-male courtship behavior and alteration in olfaction sensitivity in adult flies [292]. Painless is also expressed in the Drosophila heart and causes pauses in heartbeat, mimicking the pressure-induced response. Thus, painless might constitute part of a mechanosensitive pathway that adjusts cardiac muscle activity and mediates cardiac nociception expressed in the Drosophila heart and causes pauses in heartbeat, mimicking the pressure-induced response. Thus, painless might constitute part of a mechanosensitive pathway that adjusts cardiac muscle activity and mediates cardiac nociception [251]. Interestingly, painless isoforms contain fewer ARDs than the canonical painless protein. Those isoforms, that essentially lack ankyrin repeats, can still perform mechanical nociception, but are not involved in thermal nociception phenotypes. In contrast, isoforms that contain ARDs are sufficient for thermal nociception but are not capable of mechanical nociception [123].

Negative geotaxis in Drosophila (a movement of the fly against gravity) requires Johnston’s organ, a mechanosensory structure located in the cap cells of the antenna that also detects near-field sound. The Johnston’s organ expresses two TRPA genes, painless and pyrexia. Specific mutations of these two TRPA genes disrupt negative geotaxis [264].

Larvae of fruit flies discriminate between the optimal temperature of 18 °C and slightly higher temperatures (19–24 °C). This discrimination requires the TRPA channels that functions downstream of a PLC-dependent signaling cascade similar to that used in fly phototransduction. This signaling cascade promotes amplification of small differences in temperature and facilitates adaptation to temperatures within the comfortable range [161]. A small set of warmth-activated anterior cell (AC) neurons located in the fly brain play a critical role for temperature selection. AC neuron activation occurs just above the fly’s preferred temperature and depends on TrpA1. Flies that selectively express trpa in the AC neurons select normal temperatures, whereas flies in which TrpA1 function is reduced or eliminated choose warmer temperatures. TRPA1 promotes avoidance of slightly elevated temperatures and acts together with a distinct pathway for cold avoidance to set the fly’s preferred temperature, a general strategy for robustly selecting a narrow temperature range optimal for survival [112].

The discrimination among sensory stimuli is critical for Drosophila survival, e.g. discrimination between “noxious” chemicals or “innocuous” warming. This process requires highly tuned TRPA channels. A specific TRPA1 isoform in chemosensory neurons respond only to chemicals but not to warmth that is sensed by another TRPA isoform. Cell-type segregation of TRPA1 activity is critical: when the thermosensory isoform is expressed in chemosensors, flies respond to innocuous warming with regurgitation, a nocifensive response. Drosophila TRPA1 orthologs preferentially activated by heat have two portable heat-sensitive modules within the ARD [59]. Heat-sensing domains are encoded by specific exons of the TRPA1 locus. These exons are used for alternative splicing to produce four TRPA1 isoforms, each with distinct temperature thresholds [321].

TRPA1 isoform diversity is conserved in malaria mosquitoes, which may indicate that discrimination of host-derived warmth and chemical repellents are differentially sensed [132]. Heat sensitivity plays also a critical role in close-range host-seeking behaviors of adult female Anopheles gambiae, the principal Afrotropical vector for human malaria. The Anopheles TRPA1 channel acts as a heat-sensor in host-seeking adult female mosquitoes, and is localized at the distal antennas that specifically respond to temperature gradients [294].

Temperature discrimination via TRPA1 is also conserved in other insects and especially in the honey bee. Honey bees are relatively small heterothermic animals that are highly susceptible to changes in ambient temperature. They are able to maintain the brood nest temperature between 32 °C and 36 °C either cooling or heating the nest. The honey bee TRPA1 is activated by temperatures >34 °C as well as by insect antifeedants like camphor. It is expressed in the antennal flagellum, and ablation of the antennal flagella and injection of TRPA inhibitors impair warmth avoidance in honey bees. Gustatory responses of honey bees to sucrose are suppressed by noxious heat and insect antifeedants, but are relieved by TRPA inhibitors. Evolutionarily, honey bees lost the ancient chemical sensor that is homologous to Drosophila TRPA1. Its TRPA1 originates from duplication of the TRP channel water witch, a TRP channels providing hygrosensory together with the TRPV homolog nan [148].

Gustatory receptor neurons (GRNs) in Drosophila also express TRPA1 that responds to aversive compounds. Elimination of TRPA1 had no impact on the responses to nearly all bitter compounds tested, including caffeine, quinine, and strychnine, but is essential for the behavioral and electrophysiological responses to aristolochic acid. Aristolochic acid is rather not a direct activator or inhibitor of TRPA1, but leads to activation of PLC-dependent pathway [142]. Drosophila larvae also show food-averse migration toward food-free habitats. This developmental switching from food attraction to aversion is regulated by a neuropeptide Y (NPY)-related brain signaling peptide. A fructose-responsive chemosensory pathway, which requires painless, modulates food-averse migratory and social behaviors [307].

Taken together, these data show that TRPA1 is critically involved in insect thermal homeostasis and feeding. It is therefore tempting to suggest that the profligacy of TRPA1
modulators of plant origin has its raison-d’être in the co-evolutionary relationship between insects and plants.

Zebrafish has two TRPA1 paralogs. In this animal model the role of TRPA1 in detecting thermal and mechanical stimuli is controversial. The two zebrafish TRPA1 paralogs are expressed in sensory neurons and are activated by several chemical irritants in vitro. High-throughput behavioral analyses of trpa1a and trpa1b mutant larvae indicate that TRPA1b is necessary for behavioral responses to these chemical irritants. However, both TRPA1 paralogs are not required for behavioral responses to temperature changes or for mechanosensory hair cell function in the inner ear or lateral line. Zebrafish TRPA1 has a role in chemosensing but not in thermal or mechanical sensing [232].

TRPA1 was likely a noxious heat and chemical receptor and co-expressed with TRPV1 in the nociceptive sensory neurons of ancestral vertebrates such as Western clawed (WC) frogs and green anole lizards and later lost heat activation [242].

Recently, snake TRPA1 has been related to infrared sensing. Infrared signals are initially received by the pit organ, a highly specialized facial structure that is innervated by nerve fibres of the somatosensory system. How this organ detects and transduces infrared signals into nerve impulses is not known. Consistent with their role as primary transducers of infrared stimuli, TRPA1 orthologues from pit-bearing snakes (vipers, pythons and boas) are the most heat-sensitive vertebrate ion channels identified so far [107]. The “infrared sensory gene” TRPA1 was sequenced in 24 snake species. Pit-bearing snakes contain an 11 aa domain which is unique when compared with non-pit snakes and other vertebrates, that might be potentially functionally important for infrared sensing [101]. In another report, isoforms for infrared sensing show three parallel amino acid changes in the central region of their ARDs [311]. An intriguing question arises how the exceptional TRPA1 sensitivity functions and what is the evolutionary origin of these warmth-activated TRP channels [217].

TRPA1 in disease

TRPA1-related channelopathies

The only TRPA1 channelopathy described so far is a rare pathological pain state. The autosomal dominant Familial Episodic Pain Syndrome (FEPS) is characterized by episodes of debilitating upper body pain, triggered by fasting and physical stress. Candidate gene sequencing identified a point mutation that leads to substitution of Asn855 to Ser in S4 of TRPA1. The mutant channel has a normal pharmacology but shows a more than 5-fold increase in inward current on activation at normal resting potentials. Patients have an enhanced cutaneous flare response and secondary hyperalgesia to punctate stimuli. Specific TRPA1 antagonists inhibit the abnormal in vitro response of the mutant TRPA1 channel, promising a useful therapy for this syndrome [156, 303].

A single nucleotide polymorphism (SNP), which results in the Glu179Lys substitution, has been found in pain patients who experience paradoxical heat sensation [32]. In contrast to wild type TRPA1 expressed in HEK293 cells, which shows an higher protein expression in cold (4 °C) and heat (49 °C)-treated cells and is responsive to cold stimulation, the E179K variant is not activated by cold, possibly due to the loss of ability to interact with other proteins or defective heteromerization [186].

TRPA1 and pain

TRPA1 is an excellent candidate to explore and intricately understand how single channel properties can tailor behavioral nociceptive responses. Dermal application of electrophilic TRPA1 activators can induce spontaneous pain, heat and mechanical hyperalgesia, cold hyperalgesia and a neurogenic axon reflex erythema (vasodilation) through direct TRPA1 stimulation [96, 193, 200]. In general, also postoperative pain is mainly caused by TRPA1 activation [297]. Since it is also involved in persistent chronic painful states such as inflammation, neuropathic pain, diabetes, fibromyalgia, bronchitis and emphysema, as well as in acute noiception, it provides an excellent target for therapeutic pharmacological interventions (for a review see [8, 98]).

Peripheral diabetic neuropathy (PDN) is a major complication of diabetes mellitus (DM). Pathogenesis of PDN could be due to sustained activation of TRPA1 by ROS and RNS compounds generated in DM. In diabetic rat and mice models, the TRPA1 channel antagonist Chembridge-5861528 reduced sustained activations of the TRPA1 channel, providing a selective disease-modifying treatment of PDN [149, 296]. Similarly to DM, neuropathic pain can also develop during chronic uremia. The highly reactive compound methylglyoxal (MG) accumulates in all cells, in particular neurons, and leaks in the plasma. It is known that MG covalently modifies arginine, lysine and cysteine residues and forms advanced glycation end products, leading to hyperglycemia-induced tissue damage [40]. The electrophilic structure of the cytotoxic ketoaldehyde MG strongly suggests TRPA1 as a molecular target, also because this compound shows thiol-trapping properties. MG cause neuropathic pain in several metabolic disorders and is also considered as a promising target for medicinal chemistry [81].

Cancers cause excruciating pain that severely reduces the quality of life in cancer patients. The nerve growth factor (NGF) plays a cardinal role in inflammation and pain,
interacting with multiple pro-inflammatory cytokines. It is highly elevated in human oral squamous cell carcinoma tumors where it acts as a key endogenous molecule involved in cancer-related inflammation. NGF blockade decreases tumor proliferation and nociception. Importantly, NGF blockade also decreases the expression of TRPA1 [310]. TRPA1-caused pain is enhanced by neurotrophic factors (NTFs). Both brain-derived neurotrophic factor (BDNF) and glial cell-derived neurotrophic factor (GDNF) levels are altered in pathological pain states, and exogenous BDNF and GDNF have multiple effects on pain behavior, depending on the animal model (i.e. inflammatory vs. neuropathic). BDNF and GDNF leads to enhanced expression and activity of TRPA1 and subsequently to enhanced neuronal sensitivity to painful stimuli [57].

TRPA1 is also critically involved in pain induced by anti-cancer treatment with platinum-based anticancer drugs, oxaliplatin and cisplatin, which produces early-developing, painful, and cold-exacerbated paresthesias. Oxaliplatin and cisplatin evoked glutathione (GSH)-sensitive relaxation, mediated by TRPA1 stimulation and the release of CGRP from sensory nerve terminals, leading to either oxaliplatin-evoked mechanical and cold hypersensitivity or cisplatin-evoked mechanical allodynia [203]. Antimitotic drugs, such as paclitaxel, also cause peripheral neuropathy. Application of paclitaxel stimulated ROS formation that potentiates TRPA1 activation. These effects are prevented by N-acetyl-cysteine (a reducing agent), GSH and HC030031 [183].

The NGF receptor TrkA diversifies into peptidergic and non-peptidergic nociceptors around birth. In this process, peptidergic neurons maintain TrkA expression, while non-peptidergic neurons downregulate TrkA and upregulate the common BDNF family ligand receptor Ret and bind IB4. Ret is expressed in two distinct populations of small-medium sized non-peptidergic neurons and is a critical regulator of TrpA1. Ret-deficient mice fail to respond to TRPA1-agonist induced neurogenic inflammation [94].

TRPA1 is also involved in endothelin-1 (ET-1)-induced spontaneous pain-like behavior in mice. TRPA1 antagonists, HC-030031 and AP18, significantly reduced the pain-like behavior caused by ET-1 [169].

Another link between pain and TRPA1 comes from studies on the protease activated receptor 2, PAR2. Proinflammatory agents such as trypsin and mast cell tryptase cleave PAR2 causing activation of this receptor that is also expressed in sensory nerves. TRPA1 is functionally coupled with PAR2. Both proteins are co-localized in DRG where activation of PAR2 increased the TRPA1 currents [63].

Cytochrome-P450 (CYP450) epoxygenases metabolise arachidonic acid (AA) into four different biologically active epoxyeicosatrienoic acid (EET) regioisomers. Three of the EETs (i.e., 8,9-, 11,12- and 14,15-EET) are rapidly hydrolysed by the enzyme soluble epoxide hydrolase (sEH). sEH plays an important role in nociception during peripheral inflammation. sEH-deficient mice exhibited elevated 8,9-, 11,12- and 14,15-EET-levels, 8,9-EET sensitizes TRPA1 expressing neurons and elevates mechanical hyperalgesia. sEH knockout mice show increased nociceptive responses to mechanical stimulation in inflammation and 8,9-EET injection reduced mechanical thresholds in naive mice. Thus, sEH can regulate mechanical hyperalgesia during inflammation by inactivating 8,9-EET [34]. 5,6-EET levels increased in dorsal root ganglia (DRGs) and the dorsal spinal cord, and 5,6-EET is released from activated sensory neurons in vitro and enhances the frequency of spontaneous EPSCs (sEPSCs) in lamina II neurons in SG by TRPA1 activation. 5,6-EET presynaptically facilitated spinal cord synaptic transmission by TRPA1 causing mechanical allodynia [255].

TRPA1 is also a novel pain target in dorsal horn neurons and sensory nerve endings in the SG. Blockade of the spinal TRPA1 channel attenuates mechanical pain hypersensitivity particularly to low-intensity stimulation in various pathophysiological conditions. Blockade of the spinal TRPA1 channel reduced cutaneous neurogenic inflammation, presumably by decreasing the drive of spinal interneurons that induce a proinflammatory dorsal root reflex. Thus, the spinal TRPA1 channel also provides a promising target for development of a selective disease-modifying therapy for central pain hypersensitivity [224]. Indeed, proximal nerve endings within the spinal dorsal horn require the TRPA1 regulated transmission to spinal interneurons that is involved in pain hypersensitivity. Intrathecal administration of Chembridge-5861528 attenuates pain hypersensitivity in various experimental models (e.g. spinal nerve ligation and rapid eye movement (REM) sleep deprivation). The spinal TRPA1 channel provides a promising target for the selective attenuation of a central mechanism contributing to pathophysiologic pain [298]. Selective TRPA1 receptor antagonists were also studied in an osteoarthritic (OA) rat model. Blockade of TRPA1 disrupts transmission of high-intensity mechanical stimulation to the spinal cord in OA rats and reduces pain [187].

An important antianalgesic principle has been recently discovered. Activation of TRPA1, e.g. by electrophilic quinone metabolites of acetaminophen (paracetamol) induces depolarizations in sensory nerves, thereby inactivating voltage-dependent Na⁺ channels and attenuating action potential firing. This provides an antinociceptive effect even for some, probably slow, TRPA1 agonists [2]. Etodolac, a nonsteroidal anti-inflammatory drug, attenuates mechanical allodynia in a mouse model of neuropathic pain by a mechanism that is independent of cyclooxygenase inhibition. However, it is also shows a selective TRPA1 agonist action, providing evidence that etodolac desensitizes nociceptors or inhibiting action potential firing by selective activation of TRPA1 [125].
It is now well established that TRPA1 mediates headache by dural mechanisms. TRPA1 on meningeal nerve endings causes environmental irritant-induced headache, but is also involved in migraine. TRPA1 is expressed on a substantial fraction of dural afferents sensory nerve fibers and activation of meningeal TRPA1 produces behaviors consistent with those observed in migraine attacks. Activation of meningeal TRPA1 via endogenous or exogenous mechanisms can lead to afferent signaling and headache [82].

**TRPA1 in inflammation**

TRPA1 is a main player in the fast onset and maintenance of inflammation (Fig. 4). Many TRPA1 activating compounds are produced during inflammation as results of lipid oxidation, peroxide formation, and oxidative stress ([281], see also previous sections).

The pathogenesis of the common chronic cutaneous vascular disorder rosacea requires polymodal activation of TRPA1, including cold, pungent products from plants and spices, ROSs, and mechanical stimuli [13]. Moreover, activation of TRPA1 in the skin, e.g. in epidermal keratinocytes enhances the expression of proinflammatory cytokines, including IL-1α and IL-1β, which are known to be key contributors to skin inflammation [12].

TRPA1 activation causes the release of CGRP and substance P which are involved in the induction of neurogenic inflammation associated with the pain. Via this mechanism, TRPA1 causes vasodilation and induces edemas, a key symptom in inflamed tissue [157, 254]. Inflammation is mediated by TRPA1 via an elevation of TNFα which is a key player in joint inflammation. Blockade of TRPA1 receptors may be beneficial in reducing chronic pain in arthritis [91].

Lipogenase enzymes, such 12-LOX, generate lipid species that are involved in inflammatory hyperalgesia. In particular, hepoxilins HXA[3] and HXB[3] are increased during peripheral inflammation, and promote initiation of facilitated nociceptive processing through direct activation of TRPV1 and TRPA1 at central terminals [109].

**TRPA1 and itch**

Some irritating sensations are mediated by TRPA1, but are different from pain. Itch, the unpleasant sensation that evokes a desire to scratch, accompanies numerous skin and nervous system disorders, sometimes insensitive to antihistamine treatment. Members of the Mas-related G protein-coupled receptor (Mrgpr) family are activated by mast cell mediators and promote histamine-independent itch. MrgprA3 and MrgprC11 act upstream from TRPA1. Interestingly, TRPA1 is a target of both, MrgprA3 and MrgprC11, and is required for Mrgpr-mediated signaling. TRPA1-deficient mice display little or no scratching in response to pruritogens, indicating that TRPA1 is an essential component of the signaling pathways that promote histamine-independent itch [302].

TRPA1 induces itch upon oxidative stress. Intradermal injection of H2O2 or tert-butylhydroperoxide (tBHP) into the nape of the neck in mice induces itch which is not histamine dependent. Because resiniferatoxin treatment abolished oxidant-induced scratching, it is suggested that oxidative stress acts via C-fibers. Importantly, antioxidants and TRPA1 antagonists may be used to treat human itch conditions associated with oxidative stress [170].

![Fig. 4](image-url) Contribution of TRPA1 activation to inflammatory signaling pathways. TRPA1 and TRPV1 are sensitized or activated upon GPCR-PLC receptor activation pathways. Rise of intracellular Ca²⁺ levels through IP3-dependent ER-store release and/or TRPA1 and TRPV1 permeation is required for activation of TRPA1 and triggers release of proinflammatory neuropeptides such as substance P or CGRP. Phosphorylation through PKC and other kinases also affects TRP channel activity during inflammation (adapted from [28] with permission).
TRPA1 oro/facial/dental pain

TRPA1 plays a prominent role in the oral-dental system which is innervated by the trigeminus nerve. In a rodent model of tooth injury, TRPA1 expression is increased and contributes to hyperalgesia and allodynia [111]. Pain associated with stimulation of a sensitive tooth involves mechanotransduction. The majority of pulpal afferents express ASIC3 and TRPA1, that represent novel targets for the treatment of dentin sensitivity [116]. Odontoblasts form the outermost cellular layer of the dental pulp act as sensory receptor cells. They highly express TRPA1, which plays a key role in mediating noxious cold responses in odontoblasts [83, 84].

Vital bleaching procedures are popular means of improving the appearance of discolored teeth. These whitening products contain peroxides which come in contact with the teeth. Many users undergoing peroxide based whitening procedures complain of bleaching sensitivity (BS) arising in the treated teeth. TRPA1 is activated by the oxidizer compounds including hydrogen peroxide and direct activation of intradental nerve activity via TRPA1 [181]. Nerve Growth Factor (NGF) regulates TRPA1 by increasing its functional activity in trigeminal ganglion neurons by upregulation of its expression. This upregulation of TRPA1 likely plays an important role in the development of hyperalgesia following nerve injury and inflammation in the orofacial region [71].

TRPA1 in cardiovascular diseases

Based on the remarkable activation of TRPA1 by garlic, it is intriguing to speculate that this channel might be involved in protective effects against cardiovascular diseases [263]. However, it has been shown that the ultimate cardiovascular active compound derived from garlic is the gaseous messenger H_{2}S, which leads to vasorelaxation via K_{ATP} induced hyperpolarization in smooth muscles, prevents leukocyte adhesion, reduces cardiac arrhythmias, and acts cardioprotective (a concise review in [166]).

The effect of TRPA1 activation might be explained by changes in endothelial [Ca^{2+}], and dilation of pressurized vessels with myogenic tone. This dilation is attenuated by disruption of the endothelium and by blocking of TRPA1 with HC-030031. The AITC-induced dilation is insensitive to NOS or cyclooxygenase inhibition but requires K^{+} channels which mediate cell hyperpolarization [80]. This mechanism might also explain the beneficial effect of TRPA1 activators on high blood pressure. TRPA1 agonists significantly increase the blood flow in skin. Also, a TRPA1-dependent relaxation is present in mesenteric arteries. Intravenously-injected TRPA1 agonists induce a transient hypotensive response accompanied by decreased heart rate. These effects are not much influenced by TRPV1, CGRP or substance P. The cholinergic antagonist, atropine sulphate, inhibits the depressor response and slows heart rate caused by TRPA1 activation. Thus, TRPA1-mediated vasodilation influence changes in blood pressure possibly via the autonomic system reflexes and may contribute to the vasovagal/neurocardiogenic syncope disorders [230].

Diesel exhaust, which is emitted from on- and off-road sources, triggers adverse cardiovascular effects like arrhythmias. These arrhythmias are mediated by airway sensory nerves expressing TRPA1. TRPA1 activation causes a centrally-mediated autonomic imbalance and heightened risk of arrhythmia. Exposures of rats to diesel exhausts increase sympathetic tonus, prolong ventricular depolarization and shorten repolarization periods [115].

TRPA1 in GI tract diseases

In the esophagus, TRPA1 is present on esophageal sensory afferents and plays an important role in esophageal nociception. Both, PAR2 and TRPA1 are expressed in esophageal nodose neurons. Esophageal mast cell activation induces long-lasting mechanical hypersensitivity in vagal nodose C fibers. Mast cell tryptase via PAR2-mediated pathways sensitizes sensory nerve and induces hyperalgesia, which can be evoked by esophagus distension. TRPA1 inhibitors strikingly reduce mechanical hypersensitivity induced by either mast cell activation or PAR2 agonists [314].

TRPA1 is expressed in primary sensory afferents innervating the stomach, and is involved in visceral hypersensitivity in rats. Gastric distention induced the activation of extracellular signal-regulated protein kinase 1/2 (ERK1/2) in DRG and nodose ganglia neurons, which express TRPA1. Intrathecal injection of TRPA1 antisense RNAs attenuates the visceromotor response, and suppresses ERK1/2 activation in DRG, but not NG. The TRPA1 inhibitor HC-030031 suppresses the response to noxious gastric distention, showing that TRPA1 in primary afferents is a therapeutic target for the reduction of visceral hypersensitivity [152].

In patients with irritable bowel syndrome (IBS), stressful life events can have striking influences on visceral perception. TRPA1 is involved in the stress-induced visceral hyperalgesia, which is probably due to an upregulation of TRPA1 in the colonic afferent DRG. These neurons co-express TRPA1 and TRPV1, both considered as targets to treat the stress-induced visceral hyperalgesia [316].

Colitis is coupled to a neurogenic component involving TRPA1. Intracolonic administration of MO or trinitrobenzene sulfonic acid (TNBS) in mice leads to a severe colitis that is maintained by upregulation of TRPA1 [144, 308]. TRPA1 is expressed in DRGs projecting to the colon. Intracolonic administration of TRPA1 agonists causes expression of c-Fos in laminae I-II of the spinal dorsal horn at sacral segment S1. This results in mild colonic inflammation [48]. Activation of colonic TRPA1 channels is signaled to the spinal cord and mild colitis enhances this afferent input.
Noteworthy, neonatal colon injury results in a long-lasting visceral hypersensitivity driven by an early increase in growth factor expression and maintained by permanent changes in TRPA1 function [56].

The neuropeptides CGRP and substance-P are released from extrinsic sensory neurons, and play an important role in experimental colitis [308]. In colitis models, TRPA1 activation causes an enhanced release of colonic substance-P and CGRP. The inducer of inflammation TNBS binds covalently to cysteine and lysine residues of TRPA1. Mice with TNBS-colitis have increased colonic neuropeptide release mediated by TRPA1. Endogenous products of inflammatory lipid peroxidation also induced TRPA1-dependent release of colonic neuropeptides. Colitis induction is inhibited by TRPA1 block. Activation and sensitization of TRPA1 and release of substance-P induce and maintain colitis in mice [87].

Pancreatic inflammation is coupled to both channels in pancreatic nodose ganglia and DRG sensory neurons (identified by content of retrograde tracer). During pancreatic inflammation induced by caerulein, TRPA1 antagonists reduced caerulein-induced pain and inflammation. TRPV1 and TRPA1 inhibitors have synergistic effects. Both channels determine the excitability of pancreatic sensory neurons in vagal and spinal pathways. Specific antagonists could be developed to reduce pain in patients with acute pancreatitis [249].

TRPA1 in respiratory disease

The exposure of the upper respiratory tract to irritants alters the normal mammalian exhalation pattern, decreasing the respiration rate. This pattern of respiration rate depression has been used as an indicator of sensory irritation, and TRPA1 is at least partially responsible for this effect [245]. Activation of nasal trigeminal nerve endings by polluted air, oxidative stress or chlorine induces respiratory depression, nasal obstruction, sneezing, cough, and pain. Since TRPA1 is strongly activated by hypochlorite and hydrogen peroxide in primary sensory neurons, it seemingly acts as an oxidant detector in airway sensory neurons [30]. Chemosensory airway reflexes can provoke severe complications in patients affected by inflammatory airway conditions like rhinitis and asthma.

Coughing is the probably the most frequent reason for consultation with a physician. Endogenous inflammatory mediators such as PGE2 and BK are elevated in respiratory disease. TRPA1 expressed on respiratory vagal C fibres, often in addition with TRPV1, is a main “effector” of tussive responses to these agents. TRPA1 blockers inhibit the tussive response to PGE2 and BK, and TRAP1 is probably one of the most promising targets for the development of anti-tussive drugs [106]. However, TRPA1-mediated activation of cough can be modest compared to that mediated by TRPV1, since stimulation of TRPA1 stimulation is less efficient than that of TRPV1 to induce sustained activation of airway C-fibers [41].

Asthma is an inflammatory disorder caused by airway exposures to allergens and chemical irritants such as cigarette smoke, chlorine, aldehydes, and scents. Endogenous TRPA1 agonists, ROS, and lipid peroxidation products are other potent drivers of allergen-induced airway inflammation in asthma. In the murine ovalbumin model, the down-regulation of TRPA1 inhibits allergen-induced leukocyte infiltration in the airways, reduces cytokine and mucus production, and almost completely abolishes airway hyper-reactivity to contractile stimuli. Since this pattern of effects is also elicited by TRPA1 antagonists, TRPA1 may represent a promising pharmacological target for the treatment of asthma and other allergic inflammatory conditions. [44]. In certain subjects, an early asthmatic response (EAR) is followed by a late asthmatic response (LAR), but treatments aimed at attenuating the EAR generally fail to affect LAR. LAR evoked by allergen challenge is caused by triggering airway sensory nerves via the activation of TRPA1. This initiates a central reflex event, leading to a parasympathetic cholinergic constrictor response [234].

TRPA1 is also implicated in the pathogenesis of chronic obstructive pulmonary disease (COPD). Human lung fibroblasts and pulmonary alveolar epithelial cell express TRPA1, and its activation promotes the release of the chemokine IL-8, an effect attenuated by TRPA1 selective antagonists [196].

Thousands of persons experience accidental high-level irritant exposures each year, but most recover and few die. Persistent airway hyperresponsiveness after exposure to an irritant, gas, vapor or fumes is the clinical hallmark of asthma or reactive airways dysfunction syndrome (RADS) [39]. Many chemicals present in these irritant agents activate TRPA1 on these vagal sensory afferents and lead to central reflexes, including also dyspnea and changes in breathing pattern [26]. There are two types of cough reflexes. The mechanosensory type via olfaction and larynx movements in close proximity to the esophageal opening is mainly mediated by A fibers. The second type, the chemosensory type, has evolved to afford protection against respiratory tract infections, or the inhalation of high levels of irritant gases and particulates. This chemosensory type of reflex heavily depends on TRPA1, that becomes activated or hyperactivated after lung injury, with lung inflammation, or in response to chemicals [38]. The chemosensory cough reflex occurs via C-fibers, that cause a sensation of irritancy and an itchy urge-to-cough that mimic the urge-to-cough sensations associated with respiratory tract infection, post-infection, gastroesophageal reflux disorders, and inflammatory airway diseases [283]. Except for the action of exogenous irritants, direct TRPA1 activation in sensory nerves during cough can also occur via GPCR pathway (PGE2 and BK receptors) [178]. Blockage of TRPA1 represents a
novel therapy for the treatment of cough in humans [105, 277]. The inhalation of sulfurous vapors and thiols like cysteine or cysteamine is traditionally used to improve airways irritation [239]. It is tempting to speculate that these compounds trap endogenous activators of TRPA1 present in the inflammatory soup or, alternative, de-alkylate by sulfur exchange alkylated forms of TRPA1, restoring the channel in its native form, eventually soothing inflammation.

Finally, since TRPA1 activators are widespread in nature, the possibility exists that the inhalation of pollen, especially the one of asteraceous plants that contain highly electrophilic exomethylene lactones, might activate TRPA1, triggering inflammation and aggravating the discomfort associated to the reaction to the allergenic proteins of pollen. The gravity of symptoms associated to the allergy to asteraceous plants like *Ambrosia artemisiifolia* L. has been associated to pollution of the urban environment with ozone or acrolein, electrophilic compounds that act as TRPA1 activators [103]. On the other hand, the presence of high concentrations of electrophilic compounds in the pollen itself has been demonstrated, suggesting that activation of TRPA1 by small molecule electrophilic compounds naturally present in the pollen could add injury to the allergenic offense triggered by pollen proteins [268]. In this context, pollen might represent a vector of non-volatile electrophilic compounds to the airways. Several exomethylene-γ-lactones isolated from common ragweed are potent traps of thiol groups and activate TRPA1. Interestingly, these compounds can also activate bitter receptors [37], a type of GPCR that are highly expressed in human airway smooth muscles, potently causing relaxation [69].

TRPA1 in the urogenital system

A majority, but not all TRPA1 nerves show immunoreactivity for CGRP or TRPV1. In the urothelium, TRPV1 is located to the outer layers whereas TRPA1 is observed in basal urothelial cells. Urodynamic parameters are changed after disruption of the urothelial barrier with protamine sulfate. TRPA1, probably together with TRPV1 [168, 218, 267], is activated by H2S and participate to development of inflammatory bladder disease [261]. Additionally, acrolein, which can be formed endogenously under conditions of inflammation, aggravates this situation [190]. Intravesical TRPA1 activators also initiate detrusor overactivity that has direct clinical implications.

In chronic pain disorders, such as irritable bowel syndrome, endometriosis, fibromyalgia syndrome and also cystitis, substance P and CGRP play a decisive role. Exogenous and endogenous oxidative stress is involved in the pathogenesis of interstitial cystitis/bladder pain syndrome and other chronic pain conditions [97].

Spinal cord injury (SCI) results in tissue damage, inflammation and changes in bladder contractility and in voiding behavior. Alteration in bladder contractility following SCI is accompanied by an enhanced activity of TRPA1. SCI induced increase in the number of non-voiding contractions (NVCs), an important parameter associated with the over-active bladder (OAB) etiology, besides alterations in other urodynamic parameters. The TRPA1 antagonist HC-030031 decreases the number and the amplitude of NVCs. Thus, TRPA1 activation and up-regulation seem to exert an important role in OAB following SCI [7].

In prostate hyperplasia patients, TRPA1 is also involved in the bladder outlet obstruction (BOO). The induction of OAB by BOO might result from TRPA1 in the bladder sensory transduction via TRPA1 located in the bladder epithelium [75]. However, these findings have to be confirmed.

Conclusion and future perspectives

TRPA1 is a chemosensory channel that constitutes both an attractive drug target and a model channel for studying structural and biophysical properties. The TRPA1 gating mechanisms are still poorly understood. Although it is well accepted that reactive compounds activate the channel via covalent modification of nucleophilic sites in the N-terminus, the chemical reactions are not well understood. It is still unclear how channel activation is switched off, and second, how and which biochemical processes control viability of the channels for subsequent activation after inactivation (desensitization). Additionally, some Michael addition reactions are reversible but other covalent modifications are irreversible. We still do not completely understand the role of Ca2+ and the interference with the cytosolic Ca2+ concentrations of this highly Ca2+-permeable channel. Another urgent problem concerns the quite dramatic differences between TRPA1 channels from different species [53]. The important species dependency often concerns bimodal action of many TRPA1 modulators (e.g. menthol and caffeine). Since activation of TRPA1 can also occur via a more classical lock-and-key activation, binding sites for these non-electrophilic activators and their role as possible open pore blockers need to be clarified. Obviously, a better understanding of these mechanisms is critical for screening novel TRPA1 modulators that might be used as possible therapeu- tic agents. In this regard, it is also important to differentiate between beneficial effects of TRPA1 block versus effects of TRPA1 activation via slow depolarization and subsequent inactivation of voltage dependent Na+ or Ca2+ channels.

A unique feature of TRPA1 is its huge potential for regulation by dietary intake of channel modulators. Surprisingly, we still do not know which concentrations of these compounds are really reached in target organs and what are downstream targets of such TRPA1 activation. Many covalent and oxidant activators of TRPA1 also activate Nrf2, the master regulator of antioxidative responses. This transcription factor
causes an increased expression of antioxidant enzymes, also inhibiting inflammatory responses, and the two systems might be somewhat related, with TRPA1 acting as a sensory alert for oxidant and electrophilic insults, and Nrf2 representing instead our defense line against these agents.

It is well established that TRPA1 mediates acute and chronic pain and plays an important role in initiation but also progression and maintenance of chronic inflammatory diseases and tissue injuries, including asthma, diabetes, arthritis and skin diseases. In addition to the many effects of neuronal cells types, TRPA1 is an important channel also in non-sensory tissues, such as epithelium, smooth muscle cells, and fibroblasts. The function of TRPA1 in those cells is still poorly understood. Given the widespread potential targets for TRPA1 modulators, it is essential to understand the genetics, biophysics and physiological role of this fascinating TRPA1 channel.

Acknowledgments We thank the members of the Laboratory of Ion Channel Research for helpful discussions. This work was supported by grants from the Belgian Federal Government (IUAP P5/05), the Research Foundation-Flanders (G.0172.03, G.0565.07 and G.0149.03), and the Research Council of the KU Leuven (GOA 2004/07 and EF/95/010).

References

gating of the human TRPA1 channel. Biochim Biophys Acta 1792:1279–1288.


defining non-peptidergic nociceptor phenotypes and functions in the adult mouse. Eur J Neurosci 33:1385–1400


Pertovaara A, Koivisto A (2011) TRPA1 ion channel in the spinal dorsal horn as a therapeutic target in central pain hypersensitivity and cutaneous neurogenic inflammation. Eur J Pharmacol 666:1–4


Antinociceptive and anti-inflammatory potential of extract and isolated compounds from the leaves of Salvia officinalis in mice. J Ethnopharmacol 139:519–526


