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2 **MAMMALIAN TRANSIENT RECEPTOR POTENTIAL TRPA1 CHANNELS:**

3 **FROM STRUCTURE TO DISEASE**

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ABSTRACT

The Transient Receptor Potential Ankyrin TRPA channels are Ca^{2+}-permeable non-selective cation channels remarkably conserved through the animal kingdom. Mammals have only one member, TRPA1, which is widely expressed in sensory neurons and in non-neuronal cells (such as epithelial cells and hair cells). TRPA1 owes its name to the presence of 14 ankyrin repeats located in the N-terminus of the channel, an unusual structural feature that may be relevant to its interactions with intracellular components. TRPA1 is primarily involved in the detection of an extremely wide variety of exogenous stimuli that may produce cellular damage. This include a plethora of electrophilic compounds that interact with nucleophilic amino acid residues in the channel, and many other chemically-unrelated compounds whose only common feature seems to be their ability to partition in the plasma membrane. TRPA1 has been reported to be activated by cold, heat and mechanical stimuli, and its function is modulated by multiple factors, including Ca^{2+}, trace metals, pH, and reactive oxygen, nitrogen and carbonyl species. TRPA1 is involved in acute and chronic pain, inflammation, plays key roles in the pathophysiology of nearly all organ systems and is an attractive target for the treatment of related diseases. Here we review the current knowledge about the mammalian TRPA1 channel, linking its unique structure, widely tuned sensory properties and complex regulation to its roles in multiple pathophysiological conditions.

1. INTRODUCTION

The superfamily of *T*ransient *R*eceptor *P*otential (TRP) cation channels is composed by unique proteins that are expressed in almost every cell type, and that play key roles in diverse homeostatic functions. According to their amino acid sequence homology TRP channels are divided into seven subfamilies: TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPP ('Polycystin'), TRPML ('Mucolipin'), TRPA ('Ankyrin'), and TRPN ('NOMP-C') (226, 253, 595). One of the members of this family, TRPA1, is of special interest for being a sensor of a wide variety of noxious external stimuli such as intense cold, pungent compounds, reactive chemical species and by endogenous signals associated to cell damage. This functional diversity and its expression in nociceptive nerve fibers, epithelia and a wide variety of other cells implicate this channel in multiple diseases and make it an attractive therapeutic target.

Table 1: Clinician call-out box

TRPA1 as possible therapeutic target
TRPA1 is a Ca^{2+}-permeable cation channel activated by a wide spectrum of noxious external stimuli, such as intense cold, pungent compounds, reactive chemical species and by endogenous signals associated to cell damage.
Animal experiments indicate that TRPA1 is expressed in sensory neurons and epithelial cells and is involved in acute and chronic pain, inflammation, playing key roles in the pathophysiology of nearly all organ systems.
Further clinical research is required to evaluate the suitability of TRPA1 as therapeutic target for the treatment of peripheral and visceral hyper-sensitivity, as well as of pain and inflammatory conditions arising from the exposure to environmental pollutants and tissue injury.

Here we provide for a comprehensive review of our current knowledge on this ion channel. Given the increasing volume of publication in the field, currently more than

200 papers per year, we here restricted our focus on mammalian TRPA1 channels, but made pertinent allusions to the extremely illuminating literature on many other species, including *C. elegans*, *Drosophila*, zebrafish, etc. We may also warn the reader about the high rate of reports on newly-described TRPA1 natural and synthetic agonists and antagonists, which makes every revision on the field relatively obsolete after a few years. Finally, we would like to acknowledge the contribution of recent reviews on TRPA1, which are, because of their specialization in specific fields, or because of their completeness on specific aspects of TRPA1 properties, excellent complements to this work (60, 80, 90, 116, 210, 321, 351, 360, 410, 487, 547, 550, 557, 586, 618, 687, 691, 704, 726, 741, 746, 774, 805, 855, 899, 957, 959).

2. THE TRPA1 GENE

In humans, the *trpa1* gene is located in chromosome 8, band q21.11 and comprises 73.635 bases and 29 exons. Homologous genes have been cloned or identified in several mammal species, including 10 non-human primates, rodents, dog, cattle, pigs, etc. (Table 2). Based on structure and function, the TRPA family has only one member in mammals. The *Trpa1* gene has also been described and cloned from birds, fishes, amphibians, insects and nematodes (Table 3). Unlike mammals, some of these contain more than one gene homolog, such as *Drosophila melanogaster* (fruit fly, 4 homologues) and *Danio rerio* (zebrafish, 2).

Table 2: Mammalian TRPA1

Common name	Binomial name	GeneID	Chromosome (map location)	GNAv	Exon count
Human	*Homo sapiens*	8989	8 (q21.11)	NC_000008.11	29

Common chimpanzee	*Pan troglodytes*	464230	8	NC_036887.1	27
Sumatran orangutan	*Pongo abelii*	100460642	8	NC_036911.1	27
Western gorilla	*Gorilla gorilla*	101150478	8	NC_018432.2	27
Crab-eating macaque	*Macaca fascicularis*	102139057	8	NC_022279.1	28
Rhesus macaque	*Macaca mulatta*	694623	8	NC_027900.1	27
Northern white-cheeked gibbon	*Nomascus leucogenys*	100607287	16	NC_019831.1	27
Common marmoset	*Callithrix jacchus*	100414472	16	NC_013911.1	27
Bonobo	*Pan paniscus*	100973158	8	NC_027876.1	27
Olive baboon	*Papio anubis*	101016452	8	NC_018159.2	27
Gelada baboon	*Theropithecus gelada*	112630413	8	NC_037676.1	27
House mouse	*Mus musculus*	277328	1 (A3)	NC_000067.6	27
Gairdner's shrewmouse	*Mus pahari*	110338816	22	NC_034611.1	27
Ryukyu mouse	*Mus caroli*	110298275	1	NC_034570.1	27
Common rat	*Rattus norvegicus*	312896	5(q11)	NC_005104.4	27
Prairie vole	*Microtus ochrogaster*	101984403	LG5	NC_022031.1	27
Domestic dog	*Canis lupus familiaris*	486994	29	NC_006611.3	27
Domestic cat	*Felis catus*	101080611	F2	NC_018740.3	27
Domestic goat	*Capra hircus*	102170065	14	NC_030821.1	27
Domestic sheep	*Ovis aries*	101115717	9	NC_019466.2	29
Cattle	*Bos taurus*	505317	14	NC_037341.1	27
Horse	*Equus caballus*	100061564	9	NC_009152.3	27
Przewalski's horse	*Equus przewalskii*	103548063	Un	NW_007673276.1	26
European rabbit	*Oryctolagus cuniculus*	100341337	3	NC_013671.1	27
Wild boar	*Sus scrofa*	100152934	4	NC_010446.5	29
Water buffalo	*Bubalus bubalis*	102397027	15	NC_037559.1	27

Tibetan antelope	*Pantholops hodgsonii*	102315761	Un	NW_005812652.1	28
Polar bear	*Ursus maritimus*	103681282	Un	NW_007927247.1	28
Weddell seal	*Leptonychotes weddellii*	102730954	Un	NW_006383700.1	27
Minke whale	*Balaenoptera acutorostrata scammoni*	103012702	Un	NW_006728019.1	28
Cape golden mole	*Chrysochloris asiatica*	102826219	Un	NW_006408554.1	29
Aardvark	*Orycteropus afer afer*	103202460	Un	NW_006921768.1	27
Cape elephant shrew	*Elephantulus edwardii*	102862466	Un	NW_006399758.1	27
Gray short-tailed opossum	*Monodelphis domestica*	100028386	3	NC_008803.1	29
Tasmanian devil	*Sarcophilus harrisii*	100918272	2	N/A	Unk
Sunda flying lemur	*Galeopterus variegatus*	103585496	Un	NW_007726355.1	27
Big brown bat	*Eptesicus fuscus*	103293988	Un	NW_007370710.1	27

114 GNAv: genomic nucleotide accession version

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116 **Table 3: TRPA1 in non-mammalian species**

	Common name	Binomial name	GeneID	Chromosome (map location)	GNAv	Exon count
birds	Red junglefowl	*Gallus gallus*	420180	2	NC_006089.5	27
	Eurasian blue tit	*Cyanistes caeruleus*	111924651	2	N/A	
	Japanese quail	*Coturnix japonica*	107310278	2	NC_029517.1	27
	Great tit	*Parus major*	107214741	2	NC_031769.1	27
	Anna's hummingbird	*Calypte anna*	103527146	Un	NW_007619513.1	27
	Collared flycatcher	*Ficedula albicollis*	101813018	2	NC_021673.1	28
	Zebra finch	*Taeniopygia guttata*	100221097	2	NC_011465.1	27
	Domesticated turkey	*Meleagris gallopavo*	100545876	3	NC_015013.2	14

	Helmeted guineafowl	*Numida meleagris*	110394756	2	NC_034410.1	29
reptile	Green anole	*Anolis carolinensis*	100556580	4	NC_014779.1	30
	Green sea turtle	*Chelonia mydas*	102944221	Un	NW_006642402.1	29
	Western clawed frog	*Xenopus tropicalis*	100158526	6	NC_030682.1	27
	African clawed frog	*Xenopus laevis*	108695342	6S	NC_030735.1	27
fish	Japanese rice fish	*Oryzias latipes*	101174541	20	NC_019878.2	28
	Northern pike	*Esox lucius*	105019660	LG21	NC_025988.3	28
	Turquoise killifish	*Nothobranchius furzeri*	107382917	sgr08	NC_029656.1	27
	Mexican tetra	*Astyanax mexicanus*	103042231	3	NC_035899.1	28
	Goldfish	*Carassius auratus*	113042317	24	NC_039266.1	32
	Eastern happy	*Astatotilapia calliptera*	113028962	9	NC_039310.1	31
	Atlantic salmon	*Salmo salar*	106579725	ssa19	NC_027318.1	26
	Guppy	*Poecilia reticulata*	103456670	LG20	NC_024350.1	29
	Tongue sole	*Cynoglossus semilaevis*	103377016	3	NC_024309.1	31
	Australian ghostshark	*Callorhinchus milii*	103174784	Un	NW_006890060.1	27
	Spotted gar	*Lepisosteus oculatus*	102688457	LG9	NC_023187.1	30
	Southern platyfish	*Xiphophorus maculatus*	102223701	21	NC_036463.1	29
	Japanese puffer	*Takifugu rubripes*	101075823	10	NC_018899.1	28
	Nile tilapia	*Oreochromis niloticus*	100701720	LG9	NC_031974.2	30
	Zebrafish	*Danio rerio*	474351	2	NC_007113.7	28
insect	Red flour beetle	*Tribolium castaneum*	658860	LG3	NC_007418.3	19
	Common fruit fly	*Drosophila melanogaster*	39015	3L(3-27cM)	NT_037436.4	19
nematode	Round worm	*Caenorhabditis elegans*	178118	IV	NC_003282.8	11

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GNAv: genomic nucleotide accession version

3. STRUCTURE

The *trpa1* gene encodes a large protein, consisting of ~1100 amino acids (aa) (e.g.: 1119 aa in human, 1125 aa in rat, 1115 aa in mouse, 1120 aa in zebrafish, 1197 aa in fruit fly, 1193 aa in *C. elegans*), with an estimated molecular weight between 120 and 130 kDa.

The functional channel protein assembles in homotetramers, through 'domain-swap' interactions (178, 651). TRPA1 contains a transmembrane core conserved among the members of the TRP family, consisting of six transmembrane α-helices (TM1-6) with a re-entrant pore loop between TM5 and TM6. These two TM domains converge and form the central cavity of the channel, with two gates or restriction points. The upper gate involves diagonal interactions of opposed D915 residues, which have a functional role in Ca^{2+} permeation. The lower gate consists of two hydrophobic seals, formed by residues I957 and V961 that constrain the permeation of rehydrated cations by narrowing the funnel to ~6 Å. At the mouth of the channel, TRPA1 has two pore helices, where the negative charges of the second helix may act as the first gatekeeper by exclusively repelling anions from the gate entrance (651).

TRPA1 has distinctively large intracellular N- and C- termini, which together account for ~80% of its molecular mass (651). The long N-terminus contains between 14 and 18 ankyrin repeat domains (ARDs), each consisting of 33 amino acids (349, 358, 596, 935). The recent high-resolution 3D reconstruction of TRPA1 (at ~4 Å resolution) also provided a molecular scaffold to understand channel function (Figure 1). It revealed unexpected structural features, such as a TRP-like domain in the C-

142 terminus, directly after the TM6. Spatially, this α-helical TRP-like domain is in close
143 apposition to the pre-TM1 helix, the linker region in the N-terminus, and other non-
144 contiguous domains such as the TM4-TM5 linker, serving as a node for allosteric
145 regulation of the channel (651). TRP-domain helices, which act as a structural nexus

between the channel gates and other domains, may be a feature conserved across the entire TRP family and, possibly, other allosterically-gated channels.

Another distinctive feature of TRPA1 is the presence of a tetrameric coiled coil in the

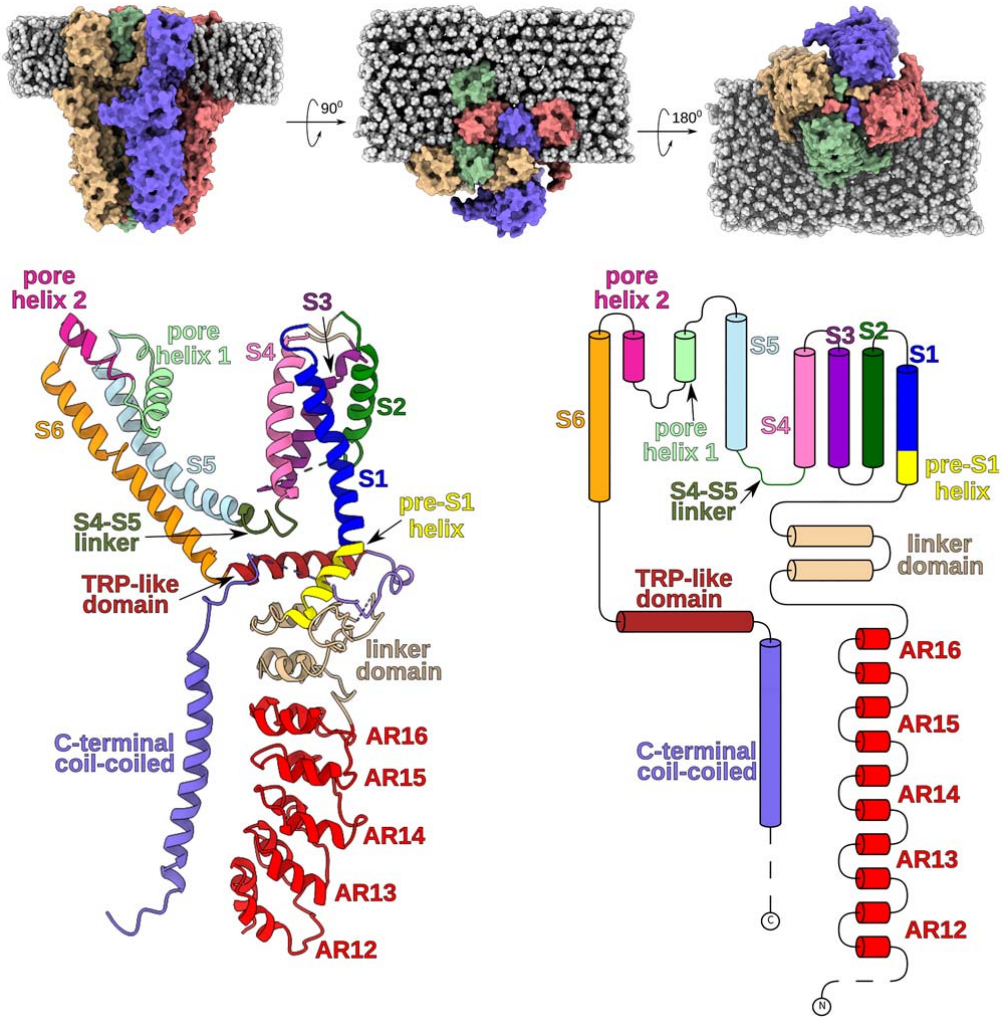

Figure 1: Structure of human TRPA1 protein. Top panels: side, extracellular and intracellular views of a three-dimensional density map of TRPA1 treated with AITC, resolved at 3.5 Å (651). Each monomer is represented with a different color. Bottom left: ribbon diagram of a hTRPA1 monomer. Bottom right: linear diagram depicting the major structural domains of TRPA1 (color-coded to match ribbon diagram). Courtesy of Dr. Ariel Talavera.

center of the channel, beneath the permeation pore. This stalk-like structure is

150 stabilized by the interaction of positively charged residues in the exterior surface of
151 the coiled coil with inositol polyphosphates (126, 651). These interactions are
152 essential for TRPA1 channel activity (388, 596, 651), suggesting that polyphosphate
153 unbinding could act as a molecular kill-switch responsible for TRPA1 inactivation.

154 Human TRPA1 features 16 ARDs, spanning for at least 150 Å. Of these, the distal 11
155 ARDs adopt a propeller arrangement that facilitate side-chain interactions with the C-
156 terminus coiled-coil region, stabilizing the ARD proximal to the plasma membrane
157 and possibly contributing to channel assembly (651). Indeed, ARD deletion results in
158 a non-functional channel protein with disturbed trafficking to the plasma membrane
159 (596). In addition, the high-resolution 3D reconstruction of human TRPA1 revealed
160 steric interactions between ARDs 12-16 and the helix-turn-helix moiety of the linker
161 region, and the regulatory TRP-like domain in the C-terminus. This web of
162 interactions possibly accounts for the regulatory effect of ARD-embedded chemical-
163 and thermal-sensitive regions on the gating properties of the channel (651).

164 Notably, the *Trpa1* gene from mouse, but not from human and rat, has two splice
165 variants (949). The shorter variant (named TRPA1b) lacks exon 20, which encodes
166 for 30 amino acids (from 777 to 807 in longer variant, TRPA1a) that span from the
167 second transmembrane domain and the first intracellular loop. Mouse sensory
168 neurons express both gene variants, but only TRPA1a was found to be a functional
169 channel according to the responses to AITC, 2-APB, thymol or carvacrol. The
170 expression of TRPA1b enhances the level of TRPA1a at the plasma membrane and
171 therefore the efficacy of chemical agonists. Intestingly, the expression of *Trpa1b*
172 mRNAs correlates with the role of TRPA1 in the late phase of mechanical
173 hyperalgesia induced by the complete Freund's adjuvant or by partial sciatic nerve
174 ligation. These findings suggest that mouse TRPA1 may be regulated through
175 alternative splicing in pathological conditions.

176 The structural analysis of TRPA1 revealed the spatial distribution of critical cysteine
177 residues within the pre-TM1 region. These residues, namely C621, C641 and C665
178 are involved in the channel activation by electrophilic compounds and its location in
179 solvent-accessible regions of the pre-TM1, suggest that covalent modifications in the
180 pre-TM1 may provide the driving force for conformational changes (178), involving
181 the neighboring subunits (i.e.: TRP-like domain) and regulating the gating properties
182 of the channel. In addition, the electron microscopy structure predicts that several N-
183 terminus cysteines form a binding pocket allowing disulfide bonding between the
184 cysteine residues. Four disulfide bonds were detected in the TRPA1 structure: C666-
185 C622, C666-C463, C622-C609 and C666-C193. In addition to the conformational
186 changes, the activation mechanism of TRPA1 may also involve disulfide bonding
187 between critical cysteine residues (867). Interestingly, as few as two subunits
188 containing intact cinnamaldehyde binding sites at position C622 are sufficient for
189 channel activation (926).

190 TRPA1 can be activated by increase in intracellular Ca^{2+} concentration $[Ca^{2+}]_i$,
191 through direct interactions of Ca^{2+} with EF hand motifs present in the N-terminus
192 domain (213). Point mutations of negatively charged residues (D466 and D477)
193 located between ARD11 and ARD12 (213, 958) abolish Ca^{2+}-dependent activation
194 (958). Deletions in this stretch, however, impairs trafficking of the truncated channel
195 to the plasma membrane (596), raising the possibility that these point mutations
196 might actually hinder proper protein expression. Another putative Ca^{2+}-binding
197 domain is formed by residues E1077, D1080, D1081 and D1082 in the distal C-
198 terminus region, which have strong effects on the Ca^{2+}- and voltage-dependent
199 potentiation and/or inactivation of agonist-induced responses. Interestingly, this
200 cluster of conserved acidic residues shows partial homology with the Ca^{2+} binding

pocket present in large conductance-Ca^{2+} activated K^+ (BK_{Ca}) channels and may constitute the long-sought Ca^{2+}-sensing domain of TRPA1 (787).

Despite the clear advances made so far, much remains to be done about structural analyses of TRPA1. Future studies should be aimed at the elucidation of the entire protein structure and at the determination of the conformational changes occurring upon stimulation with chemical agonists. This will serve, for instance, to test the hypothesis that non-electrophilic agonists may act, not by direct binding, but by inducing mechanical alterations in the plasma membrane (see below). Also important will be the identification of the binding sites of channel blockers known so far, which may be intrumental in the rational design of more potent and specific compounds of potential use in TRPA1 therapeutic targeting. Structural analyses may be also useful to unveil the mechanisms underlying several interesting features of this channel that are reviewed below, such as voltage-dependent gating, modulation by thermal stimuli, Ca^{2+}-dependent activation and inactivation and pore dilation. Importantly, these studies should consider the influence of the lipid environment and the interaction with other proteins on TRPA1 structure and function.

4. TRAFFICKING AND MEMBRANE EXPRESSION

The tumor suppressor protein CYCL interacts with TRPA1 and de-ubiquitinates the channel, thereby increasing protein levels in the cell. Oncogenic mutations of CYCL can therefore affect TRPA1 expression. Agonists induce ubiquitination of the channel and, following ligand binding, the interaction between TRPA1 and CYCL renders the channel susceptible to ubiquitination that may terminate its activation (773). Thus association of TRPA1 with the tumor-suppressor protein CYLD may become

important in oncogenic mutations in the CYLD gene, which alter cellular levels of TRPA1 (773).

The nocifensive behavior mediated by TRPA1 can be enhanced via protein kinase A/phospholipase C (PKA/PLC) signaling and by activating the channel with the ligand allyl isothiocyanate (AITC, AKA mustard oil). Both stimuli increased TRPA1 membrane levels *in vitro*. The tetanus toxin reduced the response to the second of two stimuli with AITC in neurons, indicating that vesicle fusion increases the functional expression of TRPA1 in the plasma membrane. Furthermore, capacitance recordings suggest that AITC can induce exocytosis. TRPA1 translocation to the membrane may therefore represent one of the mechanisms controlling TRPA1 functionality upon acute activation or the presence of inflammatory signals (717). Remarkably, the activation of TRPA1 by carvacrol did not increase TRPA1 trafficking. Although the experimental conditions were not identical, this suggests that the electrophilic nature of the agonist determines whether TRPA1 trafficking is induced (515).

The proinflammatory cytokine tumor necrosis factor α (TNFα) elevated the plasma membrane content of TRPA1, TRPV1 and the calcitonin gene-related peptide (CGRP). This is mediated by the vesicle-associated membrane protein 1 (VAMP1; but not 2/3), and is inhibited by botulinum neurotoxin (BoNT)/C1 or /A. Thus, these neurotoxins may act not only via the known inhibitory effect on the release of pain transmitters, but also by decreasing the exocytotic delivery of TRPA1 and TRPV1, with the concomitant reduction of hyper-sensitization during inflammation (520). Similar findings were reported using a modified chimeric BoNT, which only required pM concentrations to exert an inhibitory effect on TNFα-mediated TRPA1 and TRPV1 trafficking. This effect is dependent on the cleavage of the synaptosomal nerve-associated protein 25 (SNAP-25), confirming that TRPA1 and TRPV1 trafficking is

mediated by driven by vesicle fusion mediated by soluble N–ethylmaleimide sensitive factor attachment protein receptors (SNARE). Interestingly, this modified BoTN had no effect on basal levels of TRPA1 and TRPV1, suggesting its therapeutic potential (606). Along the same line, peripherally applied botulinum toxin type A was shown to reduce TRPA1 expression and central antinociceptive activity in rat model of trigeminal neuralgia (901). In addition to the effect of inflammation, changes of temperature have also been reported to induce TRPA1 trafficking. Both cold (4 °C) and warm (49 °C) stimuli result in higher TRPA1 expression levels (510). Future studies are requiered to elucidate whether the effects of inflammation and thermal stimuli on TRPA1 trafficking are mediated by the same pathways.

Despite our extensive knowledge about TRPA1 physiology, the molecular players and mechanisms underlying its trafficking remain largely unknown. Recent evidence suggests that TRPA1 trafficking, following activation, depends at least partially on SNARE-mediated vesicle transport. Since the effect of TRPA1 activation on trafficking is dependent on the localized influx of Ca^{2+} (717), it will be imperative to identify the Ca^{2+}-dependent mediators involved in this process and hopefully identify new potential drug targets. This approach requires a combination of pharmacological inhibitors and fluorescent live cell imaging, whereby the effect of inhibiting specific intracellular Ca^{2+}-dependent pathways on TRPA1 trafficking can be studied directly.

Regarding the expression pattern at the level of the plasma membrane, it was shown that activation of AMPK (5' AMP-activated protein kinase), an intracellular energy sensor that monitors and modulates energy expenditure, rapidly decreases membrane-associated TRPA1 and its activity within minutes. Given that high-glucose decreases AMPK activity and enhances agonist-evoked TRPA1 currents in DRG neurons, this regulation was proposed to play a role in painful diabetic neuropathy (872).

277 Disruption of lipid rafts by cleaving sphingomyelin (SM) with sphingomyelinase
278 (SMase), cholesterol depletion with methyl β-cyclodextrin (MCD) or ganglioside
279 breakdown with myriocin inhibits TRPA1 responses to AITC and formaldehyde in rat
280 trigeminal neurons (701). Furthermore, a carboxamido-steroid that disrupts lipid rafts
281 reduces TRPA1-mediated responses in Chinese hamster ovary (CHO) cells
282 transfected with the human isoform and in rat sensory neurons (699). More recently,
283 total internal reflection fluorescence microscopy and density gradient centrifugation
284 experiments revealed that TRPA1 localizes preferably into cholesterol-rich domains
285 (763). Depletion of cholesterol with an extracellular MCD treatment decreased the
286 maximal response of TRPA1 channels to AITC and reduced the sensitivity of TRPA1
287 to chemical stimulation with AITC (a 5-fold increased EC_{50}). The former effect may be
288 explained by a reduced channel expression at the plasma membrane that was
289 evidenced by confocal microscopy imaging of cells expressing mCherry-tagged
290 TRPA1 channels. The latter effect may result from the impairment of direct
291 cholesterol-TRPA1 interactions that enhance the binding affinity of AITC and/or the
292 conformational changes leading to channel opening after AITC binding. Such
293 cholesterol-TRPA1 interactions may be mediated by cholesterol recognition amino
294 acid consensus (CRAC) motifs in the TM2 and TM4 segments that are implicated in
295 the attenuation of chemical activation of TRPA1 by cholesterol-depleting agents.
296 These findings define the membrane context in which TRPA1 is expressed, which
297 may help understanding how the membrane environment may modulate the
298 responses of TRPA1, not only to chemicals, but also to mechanical and thermal
299 stimuli. Future studies should be aimed, for instance, at testing whether cholesterol-
300 TRPA1 interactions are also important for the activation of this channel by other
301 stimuli (e.g., bacterial lipopolysaccharides (LPS), cold, reactive oxygen species,
302 membrane depolarization, etc), and at determining if these interactions interfere
303 directly with the binding of chemicals or interfere with the gating machinery. In

addition, in a more translational direction, it would be interesting to assess whether cholesterol-reducing therapies using statins interfere with the sensory functions of TRPA1.

5. BIOPHYSICAL PROPERTIES OF TRPA1

5.1. GENERAL PROPERTIES OF TRPA1 CURRENTS

When evoked by a typical voltage ramp stimulus from very negative to very positive potentials whole-cell TRPA1 currents display a complex but distinctive rectification pattern. There is a slight inward rectification at very negative potentials, which changes to an outward rectification beyond around 0 mV. This behavior does not result from blocking or modulatory effects of Ca^{2+} or Mg^{2+} (864), but from an underlying voltage dependence of channel gating (491). This voltage dependence is manifested as a deactivation tail current resulting from the abrupt hyperpolarization from 0 mV, a voltage at which there is non-zero open probability. At positive potentials, the outward rectification is due to channel activation by membrane depolarization. The activation curve of TRPA1 is shifted to more negative potentials by stimuli such as intracellular Ca^{2+} (958), cold (375), menthol (371), nicotine (801), and LPS (523), rendering the channel active at physiological negative membrane potentials in sensory neurons. These shifts can be very large (e.g. 150 mV and 400 mV for the effects of intracellular Ca^{2+} (958) and menthol (371), respectively), consistent with the thermodynamic consequence of the very low apparent gating charge of TRPA1 (0.4-0.8 unitary charges (375, 597, 787, 802, 958). The negative shift of the activation curve is associated at the single-channel level to an increase in the rate of channel activation and a decrease in the rate of channel deactivation (523, 801). Interestingly, human TRPA1 is sensitized upon repetitive application of

agonists, a phenomenon that is related to a progressive negative shift of the voltage dependence of channel activation (515).

In some conditions, which remain poorly defined, the current decays at very positive potentials, a phenomenon that has been referred to as voltage-dependent inactivation (491, 596, 864). The prominence of this inactivation phase is very variable and when it is strongly manifested, outward currents are smaller than the inward currents. This phenomenon was reported to be an intrinsic property of the channel and to be mediated by the outer pore helix (864). The voltage dependence was characterized at the single-channel level in cell-attached patches with a voltage for half-maximal inactivation of +34.5 mV and an apparent gating charge of 2.4 unitary charges (slope factor = 10.9) (596).

5.2. THE PORE AND SINGLE-CHANNEL PROPERTIES

The pore of TRPA1 is formed by the selectivity filter and the S6 transmembrane segments of the four subunits of the channel tetramer. The pore diameter of the non-stimulated channel was estimated at 11 Å, according to the analysis of the relative permeability of cations of different size. Ca^{2+} binding in the pore may hinder monovalent cation permeation and carries ~17% of the mouse TRPA1 inward current. TRPA1 has therefore a relatively high fractional Ca^{2+} current and is the most Ca^{2+}-permeable amongst TRP channels. The residue D918 determines the Ca^{2+} permeation through the channel (374, 596, 876). The negatively charged residue

348 E920 in human TRPA1 and the corresponding D923 in the mouse isoform are
349 located in the outer side of the pore and may contribute to electrostatic attraction of

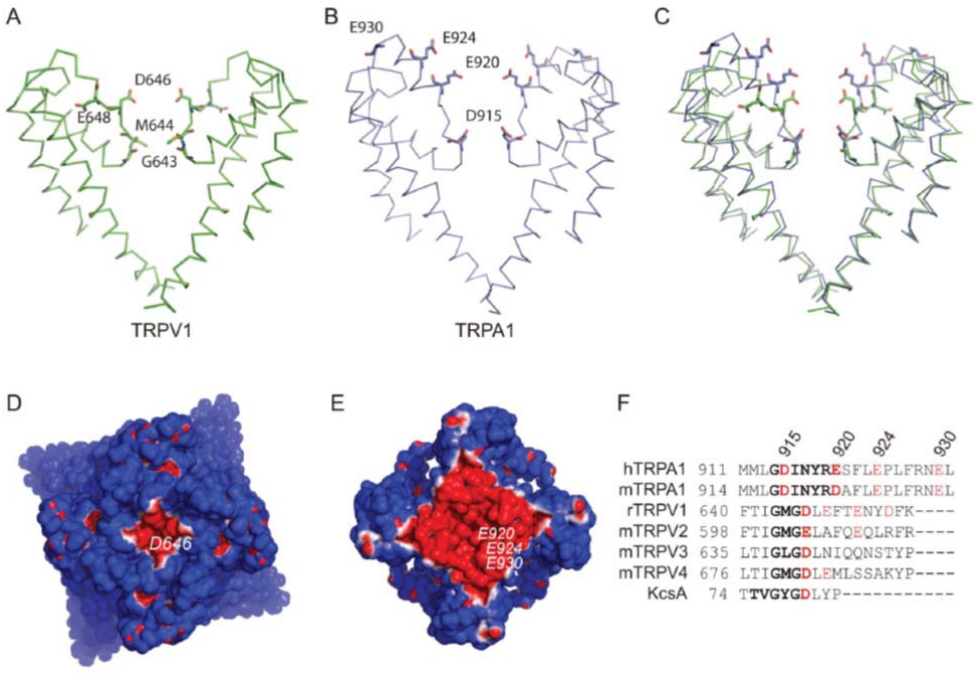

Figure 2: Positives residues in the outer pore of TRPA1 control ion permeation. (A, B) Cartoon of the pore-forming TM5 and TM6 of rTRPV1 **(A)** and hTRPA1 **(B)**. The fifth and sixth transmembrane domains and the pore loop are shown for two of four subunits. Labeled residues are stick representation and colored according to the atom type. **(C)** Alignment of the peptide backbones of TRPV1 and TRPA1. **(D)** Surface charge representation of the extracellular face of TRPV1. The color scale in electrostatic representations is from blue (1 kT/e) to red (-1 kT/e). **(E)** Extracellular face of TRPA1. **(F)** Sequence alignment of TRPA1 with the TRPV subfamily and the K^+ channel KcsA. Residues in bold form the pore based on published structures; those in red are acidic. The four acidic residues of hTRPA1 mutated in this study are at positions 915, 920, 924 and 930. Modified with permission from Christensen *et al*., (161).

350 extracellular cations to the mouth of the pore (Figure 2) (161).

351 Single TRPA1 channel properties depend on the activation mode and experimental
352 conditions (959). TRPA1 has a conductance of ~112 pS at negative and positive
353 potentials in divalent free solution, but this value is reduced to 55-65 pS at negative

potentials in the presence of extracellular Ca^{2+} and Mg^{2+}. Single mouse TRPA1 channels show subconductance states when Ca^{2+} is present in the extracellular solution (564), subconductance states were also reported for human TRPA1 in the absence of Ca^{2+} (315) or in the presence of 0.1 mM Mg^{2+} (161).

Chemical stimulation of TRPA1 induces changes in the selectivity filter that result in progressive but reversible dilation of the channel pore (66). Pore dilatation is characterized by dynamic changes in permeability to N-methyl-d-glucamine ($NMDG^+$) in (152) and an increased divalent cation selectivity and fractional Ca^{2+} current (374). Mutation of the key pore residue D918 prevents AITC-induced increases in Ca^{2+} permeation (374). TRPA1 seems to have therefore at least two open states: restricted and dilated, the latter one allowing the influx of large molecules such as Yo-Pro (Mw ~630) and $NMDG^+$. Amiloride and its analogue 5-(N,N-Dimethyl)amiloride (DMA) block more efficiently the dilated state by penetrating deeper into the channel pore (65).

TRPA1 has a relatively high Ca^{2+} selectivity, with a P_{Ca}/P_{Na} of ~6 for the constitutive open channel and ~9 for the channel activated by electrophilic agonists. The fractional Ca^{2+} current is ~17% for the constitutively open and 23% for the agonist activated channel (374). Mg^{2+} blocks the open channel but permeates at negative potentials (P_{Mg}/P_{Na} ~ 2). Ba^{2+} is also able to permeate, with P_{Ba}/P_{Na} ~3.5. The relative inorganic monovalent cation permeabilities follow the sequence $Li^+ > Na^+ > K^+ = Rb^+ > Cs^+$, with values 1.2 : 1 : 0.98 : 0.98 : 0.95. This suggest for a strong field binding cation site in the pore selectivity filter (Eisenman XI). For organic cations the sequence is Na^+ ~ dimethylamine > trimethylamine > tetramethylammonium > $NMDG^+$, with values 1 : 0.99 : 0.7 : 0.4 : 0.1. Electrophilic agonists enhance the permeability to large organic cations due to a pore dilation of 1 – 3 Å (66, 152, 374).

379 The single-channel properties of TRPA1 are modulated by co-expression with
380 TRPV1 (767). In CHO cells expressing TRPA1 the ratio of the single-channel
381 conductance values determined at positive and negative potentials was about 1.5,
382 whereas in cells co-expressing TRPA1 and TRPV1 the ratio was about 2.4. Co-
383 expression with TRPV1 also resulted in an increased open probability upon
384 membrane depolarization. These results were recapitulated when comparing single
385 TRPA1 channel properties in trigeminal ganglion (TG) neurons isolated from wild
386 type and from *Trpv1* knockout (KO) mice. Of note, co-expression of TRPV1 did not
387 change the single-channel conductance of TRPA1 when Ca^{2+} is absent in the
388 extracellular solution. In contrast, intracellular Ca^{2+} does not influence the modulation
389 of TRPA1 properties by co-expression with TRPV1 (767). Although additional
390 research is required to determine the mechanisms underlying these observations, the
391 later indicate that the functional properties of TRPA1 and therefore the
392 pathophysiological roles of this channel are regulated by the co-expression with
393 TRPV1. Key remaining questions regarding this regulation is whether it is mediated
394 by direct interactions of TRPA1 and TRPV1 homotetramers and/or by their
395 heteromerization, and whether it is modulated by the activation state of TRPV1.

396 **5.3. STRUCTURE-FUNCTION RELATION FOR GATING**

397 The mechanisms of TRPA1 gating are far from been understood because only very
398 few structure-function data are available. Residues within the TM6 inner pore-forming
399 region are implicated in activation by electrophilic compounds and in voltage-
400 dependent gating of human TRPA1. Substitution of a conserved proline residue
401 (P949) located in the middle of the TM6 by alanine strongly affect the activation and
402 deactivation. Mutation N954A results in a constitutively open channel, suggesting
403 that this residue determines the stability of the closed conformation. Alanine
404 substitutions in a distal bi-glycine motif (GXXXG) reduce the relative Ca^{2+}

permeability and affect the activation and deactivation properties. Substitution G958A leads to an increased mean open time, but not to changes in single-channel conductance. On the other hand, the mutant G962A shows short-lived and ill-resolved flickery openings at both positive and negative potentials. These findings highlight the role of the inner pore region in the control of transitions between open and closed states (87).

TRPA1 activity can be modulated by negatively charged ligands such as phosphoinositides and inorganic polyphosphates, most likely through an interaction with as yet unidentified positively charged domain(s). Twenty-seven basic residues all along the C-terminal tail of TRPA1 are implicated in activation by electrophilic compounds and voltage. Mutations of proximal C-terminus residues K969, R975, K988 and K989 affect channel function. A second significant region was found in a predicted helix, centered on K1048 and K1052. Single alanine mutations in this region completely abolish agonist- and voltage-dependent activation., The charge neutralizations K1092A and R1099A, in the distal portion of the C-terminus, reduce the sensitivity to electrophilic agonists, and increase the voltage-induced steady-state responses. This stretch of basic residues may contain possible interaction sites for negatively charged molecules that are generally considered to modulate TRPA1 (707).

The mutation N855S produces gain-of-function in TRPA1, and is associated to a familial episodic pain syndrome characterized by bouts of severe upper body pain, triggered by physical stress, fasting or cold (428, 898). It was later found that inversing charge mutation of adjacent residues E854 and K868 results in strong function decrease, whereas charge swapping recovers the channel functionality. It was proposed that these residues form inter-subunit salt bridges between adjacent

S4-S5 regions that are crucial for stabilizing the conformations associated with chemical- and voltage-dependent activation (954).

Interestingly, mutation of a conserved leucine residue, L906, in the putative pore helix results in a dramatic change in the rectification pattern of mouse TRPA1 (864). Wild type currents display an outward rectification, whereas the currents carried by the L906C mutant shows inward rectification, independent of divalent cations and irrespective to stimulation by AITC. This phenotype results from the combination of a decrease in the rate of channel deactivation and an enhanced inactivation at positive potentials. The L906C mutant is also resistant to inhibition by HC-030031 and ruthenium red.

Significant understanding of the structure-function relationship of TRPA1 was gained from single-channel recordings of hTRPA1 reconstituted into lipid bilayers (548, 549). Surprisingly, cold, heat, and electrophilic and non-electrophilic compounds activate this channel with and without its N-terminal ARD (Delta1-688 hTRPA1). Similar results were found for the inhibitory action of HC-030031. These findings demonstrate that, at least in lipid bilayers, hTRPA1 can function as an intrinsically cold-, heat- and chemo-sensitive channel (549). The N-terminal domain is also dispensable for activation of purified *Anopheles gambiae* TRPA1 by heat, AITC and cinnamaldehyde (788). On the other hand, the integrity of N-terminal ARD2, ARD6, and ARD11-13 is important for activation by chemical agonists, intracellular Ca^{2+} and membrane potential (339). Furthermore, other structure-function studies have identified structural elements in the N-terminus that when modified produce severely affected responses to thermal stimuli, including enhanced sensitivity to heat in hTRPA1 (173), distinct thermal sensitivity between splice variants of dTRPA1 (945), abrogation of cold sensitivity in mouse TRPA1 (151) and reversal of the directionality of thermally-induced activation (348). Thus, it remains possible that in physiological

456 cellular contexts the thermal and chemical sensitivities of TRPA1 channels are
457 determined by different structural elements and that the N-terminal ARD is crucial for
458 proper channel functionality.

459 A recent study proposed the existence of an intracellular water-accesible crevice
460 formed by transmembrane segments 1 to 4. Mutation of polar residues in this region
461 induced complex changes in the sensitivities to voltage, electrophilic agonists, Ca^{2+}
462 and membrane phosphoinositides (955). These changes are, however, difficult to
463 interpret unambiguously in terms of their relevance to the gating properties of the
464 channel. As was stated at the beginning of this section, the understanding of TRPA1
465 structure-function relationship is extremely limited, as for instance, the structural
466 bases of voltage-dependent gating and coupling between covalent modification by
467 electrophilic agonists to pore opening remain elusive.

468 Several other aspects of the structure-function of TRPA1 are discussed in
469 subsequent sections of this review.

470

471 **6. MODULATION, REGULATION AND ANTAGONISM OF TRPA1**

472 TRPA1 is a very attractive therapeutic target for the treatment of pain and
473 inflammation, hence the huge importance of the pharmacology of this channel.
474 However, one has to account often for striking differences between the human
475 isoform and those of rodent species. Several compounds have been identified to
476 have antagonist activity at human TRPA1, but they have large differences in potency
477 as antagonists, no effect or even

478 agonist activity in the rat and mouse isoforms. Thus, functional differences have to
479 be taken carefully into account when the modulation of this channel is considered

480 (102). Despite these differences, TRPA1 channels are arguably the most broadly-
481 tuned chemosensory channels known so far (690, 769), being activated by a wide
482 variety of chemical species (Table 4).

483 **Table 4: Agonists and bimodal modulators of mammalian TRPA1 channels**

Compound name	EC_{50} (μM)	IC_{50} (μM)	Isoform, expression system, technique	Refs.
allyl isothiocyanate	64 ± 3 11 ± 1 22 ± 3 4100 ± 800		hTRPA1, oocytes, electrophys. (+80 mV) rTRPA1, oocytes, electrophys. (-60 mV) mTRPA1, CHO, FLIPR mTRPA1, CHO, electrophys. (-75 mV)	(317) (357) (61) (241)
cinnamaldehyde	61 ± 9 250 ± 150 400 ± 40 3500 ± 300		mTRPA1, CHO, FLIPR mTRPA1, CHO, Ca^{2+} imaging hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging	(61) (22) (334)
super cinnamaldehyde	0.8		FLIPR	(491)
allicin	1.3 51 1.9 7.5 ± 0.4		mTRPA1, CHO, FLIPR rTRPA1, CHO, FLIPR hTRPA1, CHO, FLIPR hTRPA1, oocytes, electrophys. (-80 mV)	(492) (492) (492) (76)
diallyl disulfide	192 ± 3 7.6		hTRPA1, oocytes, electrophys. (-80 mV) hTRPA1, CHO T-Rex, Flex Station II	(76) (412)
diallyl sulfide	254		hTRPA1, CHO T-Rex, Flex Station II	(412)
diallyl trisulfide	0.49		hTRPA1, CHO T-Rex, Flex Station II	(412)
acrolein	5 ± 1 85 ± 9 0.8		hTRPA1, oocytes, electrophys. (-60 mV) hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(75) (334) (40)
2-chlorobenzylidene malononitrile	0.0009 0.214 0.0007		hTRPA1, HEK293-T-Rex, FDSS hTRPA1, HEK293-T-Rex, electrophys. (-30 mV) hTRPA1, HEK293-T-Rex, FLIPR	(130) (130) (609)
dibenz[b,f][1,4]oxazepine	0.0003 0.063		hTRPA1, HEK293-T-Rex, FDSS hTRPA1, HEK293-T-Rex, electrophys. (-30 mV)	(130) (130)
1-chloroacetophenone	0.03 0.275		hTRPA1, HEK293-T-Rex, FDSS hTRPA1, HEK293-T-Rex, electrophys. (-30 mV)	(130) (130)
ethyl bromoacetate	0.039		hTRPA1, HEK293-T-Rex, FDSS	(130)
bromobenzyl cyanide	0.01		hTRPA1, HEK293-T-Rex , FDSS	(130)
camphor	 ≤ 300	660	rTRPA1, HEK293, electrophys. (-80 mV) mTRPA1, CHO, Ca^{2+} imaging	(914) (22)
Δ9 tetra-hydrocannabinol	12 ± 2 0.23 ± 0.03		rTRPA1, oocytes, electrophys. (-60 mV) rTRPA1, HEK293T, Ca^{2+} imaging	(357) (192)
PF-4840184	0.097 ± 0.005 0.023 ± 0.0006		rTRPA1, FLIPR hTRPA1, FLIPR	(695) (695)
plumbagin	0.46 ± 0.05		hTRPA1, HEK293, Ca^{2+} imaging	(314)
boropinal A	10 ± 3		hTRPA1, HEK293, Ca^{2+} imaging	(314)
juglone	1.7 ± 0.5		hTRPA1, HEK293, Ca^{2+} imaging	(314)
nicotine	17	4000	mTRPA1, HEK293, electrophys. (-75 mV)	(801)
4-hydroxyhexenal	40 ± 12		mTRPA1, CHO, Ca^{2+} imaging	(35)
4-hydroxy-2-nonenal	1.9 ± 0.7		mTRPA1, CHO, Ca^{2+} imaging	(35)
4-hydroxynonenal	20 ± 3 13 27 9.9 ± 1.2 6.6 ± 1.5 6.0 ± 0.8		mTRPA1, CHO, Ca^{2+} imaging mTRPA1, CHO, Ca^{2+} imaging and FLIPR mTRPA1, HEK293T, Ca^{2+} imaging hTRPA1, HEK293T, FLIPR mTRPA1, HEK293T, FLIPR rTRPA1, HEK293T, FLIPR	(35) (491) (829) (103) (103) (103)
15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ_2)	5.6 ± 1.1 40 ± 16 60 ± 20 5.4 ± 1.1		mTRPA1, CHO, Ca^{2+} imaging hTRPA1, HEK293T, FLIPR mTRPA1, HEK293T, FLIPR rTRPA1, HEK293T, FLIPR	(35) (103) (103) (103)
hydrogen peroxide	1200 ± 400 (at 90 s) 230 (at 600 s)		mTRPA1, CHO, Ca^{2+} imaging mTRPA1, CHO, Ca^{2+} imaging	(35) (35)

Compound name	EC_{50} (μM)	IC_{50} (μM)	Isoform, expression system, technique	Refs.
	290 ± 90 297 ± 9		hTRPA1, HEK293T, Ca^{2+} imaging mTRPA1, HEK293T, Ca^{2+} imaging	(97) (714)
chloramine-T (N-chloro-sodium-p-toluenesulphenamide)	11 ± 1		hTRPA1, HEK293T, Ca^{2+} imaging	(97)
formaldehyde	357 0.016± 0.001‰ 0.015± 0.001‰		mTRPA1, CHO, Ca^{2+} imaging and FLIPR hTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(491) (513) (513)
hypochlorite	11 ± 1 ppm 7 ± 1 ppm		hTRPA1 mTRPA1, HEK293T, Ca^{2+} imaging	(97) (97)
icilin	Above 25			(211) (777)
ozone	3		hTRPA1, HEK293T, Ca^{2+} imaging	(809)
toluene diisocyanate	10000		hTRPA1, HEK293T, Ca^{2+} imaging	(807)
2-chloro-N-(4-(4-methoxyphenyl)thiazol-2-yl)-N-(3-methoxypropyl)-acetamide (JT010)	0.00065 0.047		mTRPA1, HEK293T, Ca^{2+} imaging hTRPA1, HEK293T, Ca^{2+} imaging	(797) (309)
p-benzoquinone	0.36 ± 0.02 0.44 ± 0.02 3.2 ± 0.6		mTRPA1 hTRPA1, CHO, Ca^{2+} imaging mTRPA1, CHO, electrophys. (-60 mV)	(32) (32) (32)
N-acetyl-p-benzoquinoneimine	0.9 ± 0.3 1.33 ± 0.04		mTRPA1 hTRPA1, CHO, Ca^{2+} imaging	(32) (32)
crotalphine	0.046		hTRPA1, mTRPA1, rTRPA1, COS1, Ca^{2+} imaging	(125)
methyl p-hydroxybenzoate	4400		mTRPA1, HEK293T, electrophys. (-60 mV)	(258)
3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597)	24 ± 3 70 ± 8		hTRPA1 rTRPA1, HEK293T, Ca^{2+} imaging	(591) (591)
flufenamic acid	24 ± 3 57 ± 5 44 ± 11 55 ± 4		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging hTRPA1, HEK293T, Ca^{2+} imaging hTRPA1, WI-38 fibroblasts, electrophys. (+100 mV) hTRPA1, WI-38 fibroblasts, electrophys. (+100 mV)	(334) (334) (334) (334)
niflumic acid	28 ± 3		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging	(334)
mefenamic acid	61 ± 5		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging	(334)
diclofenac	210 ± 20		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging	(334)
flurbiprofen	342 ± 6 310 ± 70		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging hTRPA1, HEK293T, WI-38 fibroblasts, electrophys. (+100 mV), Ca^{2+} imaging	(334) (334)
indomethacin	470 ± 50		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging	(334)
ketoprofen	> 500		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging	(334)
nifedipine	157 ± 8 140 ± 20 0.40 ± 0.02		hTRPA1, WI-38 fibroblasts, Ca^{2+} imaging hTRPA1, HEK293T, Ca^{2+} imaging mTRPA1, CHO, Ca^{2+} imaging	(334) (334) (245)
nimodipine	0.8 ± 1.3		mTRPA1, CHO, Ca^{2+} imaging	(245)
nicardipine	0.5 ± 0.07		mTRPA1, CHO, Ca^{2+} imaging	(245)
nitrendipine	3.8 ± 0.3		mTRPA1, CHO, Ca^{2+} imaging	(245)
(±) BayK8644	32.7 ± 0.2		mTRPA1, CHO, Ca^{2+} imaging	(245)
lidocaine	5700 ± 200 24000 ± 600		rTRPA1, HEK293T, electrophys. (-60 mV) hTRPA1, HEK293T, electrophys. (-60 mV)	(460) (460)
5-nitro-2-(3-phenylpropylamino) benzoic acid (NPPB)	0.32		hTRPA1, HEK293T, FLIPR	(481)
propofol	65.4 2.4 17	 19.5 	hTRPA1, HEK293, electrophys. (-60 mV) mTRPA1, CHO, electrophys. mTRPA1, Sf21, Flexstation III	(599) (372) (897)
thymol	64 127 20 < 100	 > 100	hTRPA1, HEK293T, Ca^{2+} imaging hTRPA1, HEK293T, electrophys. (-80 mV) hTRPA1, HEK293T, FLIPR mTRPA1, CHO, electrophys.	(458) (458) (458) (371)
menthol	95 ± 15 278 ± 30 5.2 ± 0.7 7.1 ± 1.1	56 ± 8 68 > 1000 950 ± 80 511 ± 25	mTRPA1, CHO, electrophys. mTRPA1, CHO, electrophys. (-60 mV) hTRPA1, HEK293T, FLIPR mTRPA1, HEK293T, FLIPR rTRPA1, HEK293T, FLIPR	(371) (493) (103) (103) (103)
1-hexanol	7900 ± 900		hTRPA1, HEK293T, Ca^{2+} imaging	(416)
1-heptanol	2700 ± 400		hTRPA1, HEK293T, Ca^{2+} imaging	(416)
1-octanol	810 ± 20		hTRPA1, HEK293T, Ca^{2+} imaging	(416)
apomorphine	7.1		hTRPA1, HEK293T, Ca^{2+} imaging	(719)
6-(methylsulfinyl)hexyl	150 ± 30		mTRPA1, HEK293T, electrophys. (-60 mV)	(837)

Compound name	EC_{50} (μM)	IC_{50} (μM)	Isoform, expression system, technique	Refs.
isothiocyanate (6-MSITC)	39 ± 4		hTRPA1, HEK293T, electrophys. (-60 mV)	(837)
6-(methylthio)hexyl isothiocyanate (6-MTITC)	30 ± 3 34 ± 3		mTRPA1, HEK293T, electrophys. (-60 mV) hTRPA1, HEK293T, electrophys. (-60 mV)	(837) (837)
cannabinol	0.18 ± 0.02		rTRPA1, HEK293T, Ca^{2+} imaging	(190)
cannabichromene	0.06 ± 0.02		rTRPA1, HEK293T, Ca^{2+} imaging	(192)
cannabidiol	0.096 ± 0.012 0.11 ± 0.05		rTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(192) (190)
Δ9-tetrahydrocannabinol acid	0.24 ± 0.03 2.7 ± 0.9		rTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(192) (190)
cannabidiol acid	12 ± 9 5.3 ± 1.5		rTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(192) (190)
cannabigerol	3.4 ± 1.0 0.7 ± 0.03		rTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(192) (190)
cannabigerol acid	8 ± 4		rTRPA1, HEK293T, Ca^{2+} imaging	(190)
cannabigivarin	1.60 ± 0.01		rTRPA1, HEK293T, Ca^{2+} imaging	(190)
tetrahydrocannabivarin	1.5 ± 0.6		rTRPA1, HEK293T, Ca^{2+} imaging	(190)
tetrahydrocannabivarin acid	16 ± 2		rTRPA1, HEK293T, Ca^{2+} imaging	(190)
anandamide	10 ± 2		rTRPA1, HEK293T, Ca^{2+} imaging	(191)
9-hydroxyoctadecadienoic	32 ± 4		rTRPA1, HEK293T, Ca^{2+} imaging	(191)
13-hydroxyoctadecadienoic	13 ± 2		rTRPA1, HEK293T, Ca^{2+} imaging	(191)
arachidonic acid	13 ± 4		hTRPA1, HEK293T, Ca^{2+} imaging	(679)
R-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrol[1,2,3-de]-1,4-benzoxazin-6-yl)-(1-naphthalenyl) methanone mesylate (WIN)	18 20 ± 6		mTRPA1, CHO, electrophys. (-60 mV) rTRPA1, HEK293T, Ca^{2+} imaging	(11) (751)
(R,S)-3-(2-iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinylmethyl)-1H-indole (AM1241)	48		mTRPA1, CHO, electrophys. (-60 mV)	(11)
N-(2-chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide (ACEA)	12		mTRPA1, CHO, electrophys. (-60 mV)	(11)
AM251	0.86 ± 0.06		rTRPA1, HEK293T, Ca^{2+} imaging	(751)
AM630	1.9 ± 0.2		rTRPA1, HEK293T, Ca^{2+} imaging	(751)
deacylasadisulfide propionate	11.0 ± 1.4		rTRPA1, HEK293T, Ca^{2+} imaging	(739)
deacylasadisulfide arachidate	11.0 ± 1.4		rTRPA1, HEK293T, Ca^{2+} imaging	(739)
asadisulfide alcohol	10.9 ± 0.8		rTRPA1, HEK293T, Ca^{2+} imaging	(739)
foetisulfide A	11 ± 4		rTRPA1, HEK293T, Ca^{2+} imaging	(739)
isovelleral	0.50 ± 0.13 2.6 ± 1.1		hTRPA1 mTRPA1, HEK293T, Ca^{2+} imaging	(240) (240)
polygodial	0.40 ± 0.07 0.059 0.67		hTRPA1, HEK293T, Ca^{2+} imaging hTRPA1, CHO, Ca^{2+} imaging mTRPA1, HEK293T, electrophys. (-60 mV)	(240) (240) (240)
miogatrial	0.13 0.63		hTRPA1, CHO, Ca^{2+} imaging mTRPA1, HEK293T, electrophys. (-60 mV)	(240) (240)
miogadial	0.2 0.4		hTRPA1, CHO, Ca^{2+} imaging mTRPA1, HEK293T, electrophys. (-60 mV)	(240) (240)
crotonaldehyde	23		rTRPA1, HEK293T, Ca^{2+} imaging	(40)
hydroxy-α-sanshool	69		hTRPA1, HEK293T, Ca^{2+} imaging	(681)
6-shogaol	11.2 16 ± 2	 16.7 ± 0.4	hTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(681) (552)
6-paradol	71		hTRPA1, HEK293T, Ca^{2+} imaging	(681)
linalool	117		hTRPA1, HEK293T, Ca^{2+} imaging	(681)
carvacrol	750 ± 110 7		WC frog TRPA1, oocytes, electrophys. (-60 mV) hTRPA1, HEK293T, FLIPR	(703) (458)
eugenol	260		hTRPA1, HEK293T, electrophys. (-60 mV)	(164)
1'S-1'-acetoxychavicol acetate	0.16		hTRPA1, HEK293T, Ca^{2+} imaging	(576)
2-tert-butyl-5-methylphenol	3		hTRPA1, HEK293T, FLIPR	(458)
2,6-dimethylphenol	31		hTRPA1, HEK293T, FLIPR	(458)
2,5-dimethylphenol	57		hTRPA1, HEK293T, FLIPR	(458)
3,4-dimethylphenol	67		hTRPA1, HEK293T, FLIPR	(458)
2,6-diisopropylphenol	4		hTRPA1, HEK293T, FLIPR	(458)
caffeine	96 ± 11 62 ± 3 1000-2500	 990 ± 120	rTRPA1 mTRPA1 hTRPA1, HEK293T, FLIPR mTRPA1, HEK293T, Ca^{2+} imaging	(103) (103) (566) (566)
trinitrophenol	107 ± 6 30 ± 5		hTRPA1, HEK293T, FLIPR mTRPA1, HEK293T, FLIPR	(103) (103)

Compound name	EC_{50} (μM)	IC_{50} (μM)	Isoform, expression system, technique	Refs.
farnesyl thiosalicylic acid	4.9 ± 0.9 86 ± 13 100 ± 10		hTRPA1, HEK293T, FLIPR mTRPA1, HEK293T, FLIPR rTRPA1, HEK293T, FLIPR	(103) (103) (103)
3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597)	8 ± 2 74 ± 20 129 ± 23		hTRPA1, HEK293T, FLIPR mTRPA1, HEK293T, FLIPR rTRPA1, HEK293T, FLIPR	(103) (103) (103)
4-methyl-N-[2,2,2-trichloro-1-(4-nitro-phenylsulfa-nyl)-ethyl]-benzamide (CMP1)	0.93 ± 0.05 0.88 ± 0.03	 1.0 ± 0.1 2.7 ± 0.3	mTRPA1, HEK293T, FLIPR rTRPA1, HEK293T, FLIPR hTRPA1, HEK293T, FLIPR rhTRPA1, HEK293T, FLIPR	(103) (103) (103) (103)
6-gingerol	10.4 ± 0.03	> 100	rTRPA1, HEK293T, Ca^{2+} imaging	(552)
L-carveol	190 ± 30		hTRPA1, Flp-In 293, Ca^{2+} imaging	(546)
trans-p-methoxycinnamaldehyde	30 ± 15		hTRPA1, Flp-In 293, Ca^{2+} imaging	(546)
methyl eugenol	160 ± 20		hTRPA1, Flp-In 293, Ca^{2+} imaging	(546)
4-allylanisole	1500 ± 300		hTRPA1, Flp-In 293, Ca^{2+} imaging	(546)
p-anisaldehyde	550 ± 70		hTRPA1, Flp-In 293, Ca^{2+} imaging	(546)
piperine	30		hTRPA1, HEK293T, Ca^{2+} imaging	(628)
isopiperine	33		hTRPA1, HEK293T, Ca^{2+} imaging	(628)
isochavicine	71		hTRPA1, HEK293T, Ca^{2+} imaging	(628)
piperanine	150		hTRPA1, HEK293T, Ca^{2+} imging	(628)
piperolein A	7.8		hTRPA1, HEK293T, Ca^{2+} imaging	(628)
piperolein B	11		hTRPA1, HEK293T, Ca^{2+} imaging	(628)
N-isobutyl-(2E,4E)-tetradeca-2,4-diamide (N-tetra)	19		hTRPA1, HEK293T, Ca^{2+} imaging	(628)
curcumin	3.3		rTRPA1, HEK293T, Ca^{2+} imaging	(573)
oleocanthal	2.8		hTRPA1, HEK293T, electrophys. (-60 mV)	(657)
umbellulone	19 ± 4 28 ± 7	 410 ± 50	rTRPA1, HEK293T, Ca^{2+} imaging hTRPA1, HEK293T, Ca^{2+} imaging mTRPA1, CHO, electrophys.	(583) (583) (943)
dihydroumbellulone	22	340 ± 80	mRPA1, CHO, electrophys.	(943)
tetrahydroumbellulone	ND	380 ± 30	mTRPA1, CHO, electrophys.	(943)
β-umbellulol	ND	420 ± 40	mTRPA1, CHO, electrophys.	(943)
acetyl tetrahydroumbellulone	ND	490 ± 60	mTRPA1, CHO, electrophys.	(943)
acetyl β-umbellulol	ND	> 1000	mTRPA1, CHO, electrophys.	(943)
ligustilide	44	1500	mTRPA1, CHO, electrophys.	(944)
dehydroligustilide	540	23	mTRPA1, CHO, electrophys.	(944)
capsiate	2.76 ± 0.08		hTRPA1, HEK293T, electrophys. (-60 mV)	(738)
dihydrocapsiate	2.9 ± 0.2		hTRPA1, HEK293T, electrophys. (-60 mV)	(738)
nordihydrocapsiate	2.82 ± 0.16		hTRPA1, HEK293T, electrophys. (-60 mV)	(738)
artepillin C	1.8		hTRPA1, HEK293T, Ca^{2+} imaging	(305)
baccharin	16		hTRPA1, HEK293T, Ca^{2+} imaging	(305)
drupanin	> 250		hTRPA1, HEK293T, Ca^{2+} imaging	(305)
methyl syringate	510		hTRPA1, Flp-In 293, Ca^{2+} imaging	(757)
perillaldehyde	41 ± 8 42 ± 8		rTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(73) (72)
perillaketone	22 ± 2 20 ± 2		rTRPA1, HEK293T, Ca^{2+} imaging rTRPA1, HEK293T, Ca^{2+} imaging	(73) (72)

6.1. ELECTROPHILIC ACTIVATORS

Due to their reactivity, exogenous and endogenous electrophilic TRPA1 agonists modify the thiol group of cysteine as well as lysine residues in the N-terminus of TRPA1 (317, 491, 793). In the case of human TRPA1, these were reported to be cysteine residues at positions C619, C639, and C663 and to a lesser extent lysine 708, all located in the cytoplasmic N-terminal tail of the channel between the last

ankyrin repeat and the first transmembrane segment (317). Mutation of the three cysteines results in weaker responses to electrophilic compounds, but preserves the responsiveness to the non-electrophilic agonists Δ9-tetra-hydrocannabinol and 2-APB (317). Mutation of a lysine residue (K708Q) was shown to abrogate the residual response of the triple cysteine human TRPA1 mutant to AITC.

Immunoprecipitated mouse TRPA1 can be covalently modified by electrophilic agonists, and at least 14 other cysteine residues may contribute to the activation of the channel (491). The key cysteine residues of mouse TRPA1 (C415, C422, and C622) are close to or within the ankyrin repeats, illustrating intriguing differences with the human isoform (491). Cysteine residues located in the mouse TRPA1 N-terminal can form disulfide bridges (959) that can be shared (e.g., 666–C622, C666–C463, C666–C193, and C622–C609), suggesting that channel activation may involve different N-terminal conformations (340, 867). Additionally, mass spectrometric analysis of TRPA1 showed that many of these critical cysteines (C193, C415, C463, C622, C634, and C666), along with some other cysteines (C31, C45, C66, C89, C105, C214, C259, C274, C541, C609, and C1087) are modified upon exposure to electrophiles. This suggests that electrophilic binding could lead to disruption of some existing disulfide bonds, as well as to the formation of new such bonds (708). The recent cryo-EM structure revealed the location of cysteine and lysine residues in the transmembrane core and facing the lipid environment, which may react with lipophilic electrophiles (651). Currently, it is still unknown what specific cysteine residues, of a total of 28 in human TRPA1, are critical for gating by different electrophiles. Therefore, the mechanism of channel activation after cysteine modification is yet to be fully clarified.

Of note, it was reported that electrophilic compounds can induce hTRPA1 activity in the absence of the N-terminal ANK repeats (549). It was also suggested that the

517 ARD, the pre-S1 helix, the TRP-like domain, and the linker regions of the channel are
518 vital for conformation changes that lead to activation by electrophilic compounds
519 (708).

520 The class of electrophilic chemical activators of TRPA1 includes, among others,
521 isothiocyanates (the pungent compounds in mustard oil, wasabi, and horseradish)

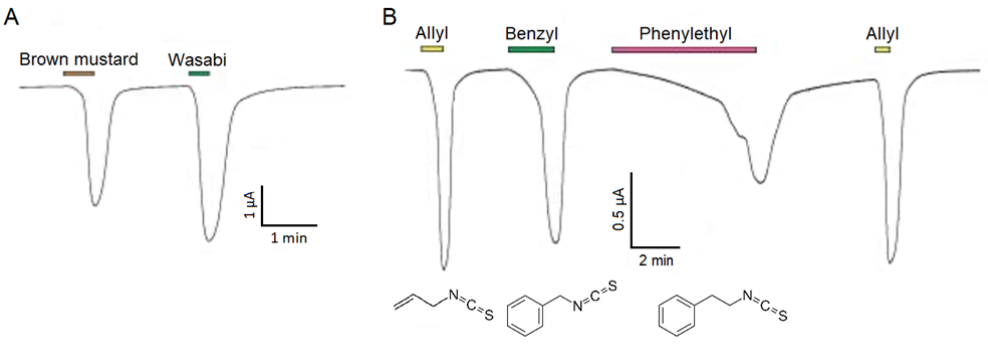

Figure 3: Activation of TRPA1 by electrophilic compounds. Activation of human TRPA1 currents in oocytes by natural extracts of brown mustard, wasabi **(A)**, allyl-, benzyl- or phenylethyl isothiocyanate **(B)**. Agonist structures are indicated below each current trace. Modified with permission from Jordt *et al*., (357).

522 (61, 357) (Figure 3), methyl salicylate (in winter green oil) (61), cinnamaldehyde (in
523 cinnamon) (61) allicin and diallyl disulfide (in garlic) (75, 492), acrolein (an irritant in
524 vehicle exhaust fumes and tear gas) (75), chlorobenzylidene malononitrile (44),
525 benzylidenemalononitriles (477), Δ9 tetra-hydrocannabinol (Δ9THC, the psychoactive
526 compound in marijuana (357, 777), chalcones (558), phenylpropanoids (boropinal),
527 1,4-naphthoquinones (juglone, plumbagin) (314), epoxyeicosatrienoic acids (5,6-
528 EET) (745), and (E)-2-alkenals (109).

529 Sulfhydryl reacting agents that modify cysteines at an N-terminal site between the
530 last ankyrin repeat and TM1 are necessary for this chemical reactivity (in human
531 C619, C639, C663) (317). These include cinnamaldehyde (CA), super
532 cinnamaldehyde (SC), SC-alkyne (SCA), acrolein, pental, AITC, mustard oil alkyne

(MOA), iodoacetamide (IA), IA-alkyne (IAA) and 2-aminoethyl methanethiosulfonate hydrobromide (MTSEA, used for cysteine scanning). The carbon chain length of isothiocyanates is not a main determinant of their ability to activate TRPA1 (815). In mouse TRPA1, out of 31 cysteine residues, the most reactive residues are C415, C422 between 10^{th} and 11^{th} ARDs) and C622 (between last ARD and TM1). Covalent modification can cause sustained TRPA1 activation (491, 655). Although it is now generally accepted that TRPA1 is activated through covalent modification of specific cysteine residues, the precise mechanism and the chemistry of this covalent modification with unsaturated carbonyl-containing compounds remain unclear. Channel activation occurs with chemicals that could only react with cysteine residues via conjugate addition such as acrylamide, acrylic acid and cinnamic acid. These compounds react via either conjugate or direct addition, such as acrolein, methyl vinyl ketone, mesityl oxide, acrylic acid N-hydroxysuccinimide (NHS) ester, cinnamaldehyde and cinnamic acid NHS ester. Direct addition occurs via propionic acid NHS ester and hydrocinnamic acid NHS ester. These reaction schemes suggest that TRPA1 is activated preferentially by direct addition of the thiol group of TRPA1 cysteines to the agonist carbonyl carbon of unsaturated carbonyl-containing compounds (696). Unraveling of the chemistry behind activation and deactivation of TRPA1 via covalent modifications remains an exciting challenge.

TRPA1 activity is modulated by O_2 in a complex manner. A stimulatory action of hyperoxia is mediated by glutathione-sensitive oxidation of cysteine residues C633 and C856 located in the N-terminus of the channel (792).

Notably, human TRPA1 is also activated by hypoxia via a decrease in the activity of prolyl hydroxylases (PHDs) and the resulting reduction of the tonic inhibition of the channel induced by hydroxylation of the N-terminal residue P394. Hypoxia also increases the insertion of nonhydroxylated channels in the plasma membrane.

559 TRPA1 is therefore activated by both hypoxia and hyperoxia, displaying minimal
560 basal activity at O_2 concentrations close to the physiological values. Interestingly, this
561 type of modulation may impact of the activation of the channel by several factors.
562 These include cold (because O_2 levels in solution increase at lower temperatures),
563 Zn^{2+} (because this ion interacts with cysteine residues) and the function of
564 mitochondria (because these organelles consume the majority of cellular O_2 and
565 hence regulate the concentration of this gas in the cytosol). Thus, O_2 seems to be yet
566 another physiological tuning factor of TRPA1 activity.

567 H_2S is one of the most important TRPA1 activators (617). NaHS, a donor of H_2S,
568 activates TRPA1, but not TRPV1. H_2S may be involved via this pathway in several
569 physiological processes including nociception (535), as this gas functions as an
570 endogenous transmitter in humans. TRPA1 activation by H_2S seems to underlie the
571 vasoactive effects of this compound, as TRPA1 mediates the release of CGRP from
572 sensory nerves of rat tracheae and to cutaneous vasodilatation in the mouse ear
573 (670). TRPA1 receptor activation should be considered as a potential mechanism of
574 H_2S. Furthermore, polysulfides (H_2S_n) produced by the interaction between H_2S and
575 nitric oxyde (NO) also activate TRPA1 (534). Interestingly, dimethyl trisulfide induces
576 analgesia via activation of TRPA1 and the release of somatostatin and its
577 subsequent action on sst4 receptors (74, 671).

578 H_2S increases cAMP levels in neuronal and glial cell lines and primary neuron
579 cultures with hyperpolarization. In addition to its action on TRPA1, H_2S may be
580 involved in multiple signaling pathways and produce various effects on ion channels
581 (e.g. T-type Ca^{2+} channels, ATP-sensitive K^+ (K_{ATP}) channels), which may inhibit or
582 promote nociception. It is also conceivable that H_2S may affect the N-methyl-d
583 aspartate (NMDA) receptor complex (748). Activation of $Ca_V3.2$ contributes to the
584 H_2S-induced mechanical hyperalgesia and allodynia in mice. Thus, T-type Ca^{2+}

channel and TRPA1 channel blockers may be an efficient way to attenuate NaHS/H_2S-induced mechanical hyperalgesia and allodynia (627). The functional link between these channels is also evidenced in the context of H_2S-induced colonic pain and referred hyperalgesia in mice (833). Dual effects of H_2S are also found in rat pancreatic islet-derived cells (RIN14B), which express K_{ATP} and TRPA1 channels. It was proposed that activation of K_{ATP} channels by the H_2S donor NaHS reduces spontaneous oscillation of $[Ca^{2+}]_i$, whereas activation of TRPA1 results in a delayed sustained increase in $[Ca^{2+}]_i$ (839).

In general, most of ROS (reactive oxygen species, such as peroxide causing cysteine oxidation), RNS (reactive nitrogen species, such as NO mediating S-nitrosylation) and RCS (reactive carbonyl species, such as PGJ2 and unsaturated aldehydes mediating cysteine carbonylation) behave as TRPA1 activators (425, 737, 794). The typical ROS, H_2O_2 is contained in industrial products and also generated within cells, and induces pain and activates TRPA1. The effects of H_2O_2 on TRPA1 are mimicked by other ROS and by RNS. Cysteine-reducing agents suppress H_2O_2-induced TRPA1 activation, whereas cysteine-oxidizing agents activate TRPA1 (714). Activation by H_2O_2 is also inhibited by scavengers the hydroxyl radical such as the tyrphostin AG-related compounds AG555 and AG556 (818). Oxydative stress leads to the production of oxidized phospholipids (OxPAPC) that are able to activate TRPA1 via cysteine modification, leading to acute pain, hyperalgesia and induced pro-nociceptive peptide release (479, 614).

Among RNS, nitric oxide is a potent TRPA1 activator. NO 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 guanosine monophosphate (cGMP) pathway and in the periphery on sensory neurons through direct activation of TRPA1 (and also TRPV1). Tetrahydrobiopterin

611 (or BH4) is an essential co-factor for NO production that stimulates a subset of dorsal
612 root ganglion (DRG) neurons by TRPA1 activation (536). Nitro-oleic acid (9-OA-NO_2),
613 an electrophilic fatty acid byproduct of NO and nitrite reactions is a potent TRPA1
614 activator (79). Excessive nitric oxide during inflammation (nitrative stress), leads to
615 the nitration of phospholipids resulting in the formation of this highly reactive cysteine
616 modifying agents. 9-OA-NO_2 fails to activate TRPA1 in which the cysteines at
617 position C619, C639, C663 and the lysine at 708 are mutated (806). The effects of
618 OA-NO_2 are blocked by dithiothreitol, but cannot be prevented or reversed by the
619 NO-scavenger carboxy-PTIO (2-(4-carboxyphenyl)-4,4,5,5-tetramethylimidazoline-1-
620 oxyl-3-oxide) (723). N-nitroso-2-exo,3-exo-ditrifluoromethyl-7-
621 azabenzobicyclo[2.2.1]heptane (NNO-ABBH1) S-nitrosylates TRPA1 and activates
622 this channel, being this effect sensitive to specific cysteine mutations but not to
623 scavenging of NO (424, 426).

624 The list of TRPA1 agonists comprises also electrophilic prostaglandins (PG). The
625 PGD2 metabolite 15-deoxy-delta(12,14)-prostaglandin J2 (15dPGJ2) activates
626 human TRPA1 expressed in human embryonic kidney (HEK) cells and in mouse
627 trigeminal neurons (176). This effect is blocked with ruthenium red and the selective
628 TRPA1 inhibitor HC-030031. This effect is not mimicked by their non-electrophilic
629 precursors, PGE2 and PGD2, or PGB2, which differs from PGA2 only in that its
630 electrophilic carbon is rendered unreactive through steric hindrance. Nonetheless,
631 TRPA1 is required for the nociceptive effects of PGE2 in vivo (181).

632 The analgesic action of nonsteroidal anti-inflammatory drugs (NSAIDs) results from
633 inhibition of cyclooxygenases and blockade of PG biosynthesis. Cyclopentenone
634 PGs, 15-d-PGJ2, PGA2, and PGA1, formed by dehydration of their respective parent
635 PGs, PGD2, PGE2, and PGE1, possess a highly reactive unsaturated carbonyl
636 group required to activate TRPA1. Cyclopentenone PGs produce pain by direct

637 stimulation of nociceptors via TRPA1 activation. Some PGs are proalgesic. Thus,
638 TRPA1 antagonism may contribute to suppress pain evoked by PG metabolites
639 without the adverse effects of inhibiting cyclooxygenases (508).

640 TRPA1 is also activated by alkylating compounds such as the chemical warfare
641 agent sulfur mustard (SM) and 2-chloroethyl-ethylsulfide (CEES) (772). N-acetyl-L-
642 cysteine, but not glutathione, prevents activation of human TRPA1 by SM,
643 suggesting that the former interects directly with the channel (771). CEES-induced
644 cytotoxicity and Ca^{2+} responses in human A549 lung epithelial cells are reduced by
645 AP18. It was therefore proposed that TRPA1 inhibition may serve to reduce SM-
646 induced cell damage. In this line, a later study showed that mouse skin lesions
647 induced by CEES are reduced by the TRPA1 inhibitors HC-030031 and A-967079
648 and a by the CGRP inhibitor MK-8825 (2).

649 TRPA1 activators can be generated during issue damage. 4-hydroxynonenal (or
650 trans-4-hydroxy-2-nonenal or 4-HNE or HNE) is an α,β-unsaturated hydroxyalkenal
651 produced by lipid peroxidation in cells. HNE is found in higher quantities during
652 oxidative stress in inflamed tissues due to the increase in the lipid peroxidation chain
653 reaction. HNE acts as an endogenous agonist for TRPA1 and promotes acute pain,
654 release of substance P and CGRP from nerve endings and neurogenic inflammation.
655 HNE acts via covalent modification of the cysteine and lysine residues in the TRPA1
656 N-terminus because quadruple mutations TRPA1-3C/K-Q mutants are insensitive to
657 HNE (829). Endogenous HNE effects are abolished with dithiothreitol (DTT),
658 indicating that they act by formation of disulfide bonds. In contrast, the actions of
659 alkenyl aldehydes and 15d-PGJ2 are not reversed by DDT, suggesting that these
660 agents form Michael adducts (35). Oxidative stress, a pathological feature of many
661 respiratory diseases, causes the endogenous formation of a number of reactive

662 electrophilic alkenals via lipid peroxidation, such as alkenal, HNE and another
663 alkenal, 4-oxononenal, which is far more electrophilic than HNE (808).

664 TRPA1 is activated by several lipid compounds (farnesyl thiosalicylic acid, farnesyl
665 thioacetic acid and 5,8,11,14 eicosatetraynoic acid) and two marketed compounds:
666 disulfiram (Antabuse; a compound used in the treatment of alcohol abuse) and the
667 anti-fungal agent chlordantoin (496). Also constituents of tear gases induce TRPA1
668 activation. The release of methyl isocyanate in Bhopal, India, caused the worst
669 industrial accident in history (99). Other component of tear gas such as 1H-
670 dibenz[b,e]azepines (morphanthridines) and dibenz[b,f][1,4]oxazepines are highly
671 potent TRPA1 activators (4, 275).

672 It is widely known that inhalation of ozone is a major health risk in industrialized
673 countries and impairs lung function through sensory neural-mediated pathways
674 (vagal nociceptive C type bronchopulmonary nerves). Ozone stimulates TRPA1 but
675 not TRPV1 (809). It must be noted, however, that no evidence of TRPA1 involvement
676 in ozone-induced cough hyperresponsiveness was found in rabbit or guinea pig
677 (167).

678 Formalin causes pain and is therefore widely used in animal models for testing the
679 effects of analgesics (250). Although formalin directly activates TRPA1 (513), this
680 compound also induces Ca^{2+} release from intracellular stores in sensory neurons,
681 primary keratinocytes and in non-neuronal cell lines in a TRPA1-independent
682 manner. This mechanism may underlie formaldehyde-induced neuronal excitation
683 and subsequent inflammation (252).

684 Toluene diisocyanate is used as a chemical intermediate in the production of
685 polyurethane products such as foams, coatings, and elastomers. It is a reactive
686 hazardous irritant that causes respiratory symptoms such as cough, rhinitis, dyspnea

687 and chest tightness in exposed workers. TRPA1 was found to be activated by this
688 compound (206, 807), and its inhibition to reduce airway hyperreactivity, neutrophilia
689 and eosinophilia, and Th2-mediated responses (924).

690 A recent screening study identified three electrophilic molecules sharing a 2-chloro-
691 N-(thiazol-2-yl)acetamide structure as very potent agonists of TRPA1, with effective
692 concentrations below 1 nM. One of these compounds, 2-chloro-N-(4-(4-
693 ethoxyphenyl)thiazol-2-yl)-N-(3-methoxypropyl)acetamide, named JT010 is
694 ineffective on TRPV1, TRPV3, TRPV4, TRPM2, TRPM8 and TRPC5 up to 1 μM
695 (797) and has been proposed as a model to trigger TRPA1-mediated pain in humans
696 (308). In a series of experiments using a biotinylated analogue of JT010, this
697 compound activated TRPA1 by binding covalently to the residue C621 (797). This
698 particular cysteine residue was shown to have an exceptionally high reactivity that
699 largely surpasses that of antioxidant enzymes (58).

700 Capsazepine, the renowned TRPV1 inhibitor, activates TRPA1 via cysteine
701 modification and exerts a strong desensitizing effect on the channel. Notably, TRPA1
702 is required for the anti-inflammatory and anti-nociceptive effects of systemic
703 administration of capsazepine, suggesting for a novel therapeutic strategy against
704 inflammation and pain (394).

705 Interestingly, also animal-produced electrophiles are able to activate TRPA1. The
706 electrophilic arthropod defensive compound, para-benzoquinone (pBQN) potently
707 activates human TRPA1 (from 10 nM), with the critical implication of three cysteine
708 residues (C621S, C641S, C665S) that are distinct from reported to be crucial for the
709 action of other electrophiles. At concentrations higher than 300 nM the activatory
710 effect is reduced and is followed by a very fast desensitization phase, which is also
711 dependent on the above-mentioned cysteine residues (340). Another animal-derived

712 TRPA1 agonist is crotalphine, a structural analogue of an analgesic peptide found in
713 the venom from the South American rattlesnake *Crotalus durissus terrificus* (125).
714 Crotalphine activates the mouse, rat and human TRPA1 isoforms with low efficacy
715 but high affinity, requiring specific cysteine residues in the N-terminal of the channel.
716 Importantly, crotalphine produces a strong desensitization to subsequent application
717 of AITC or carvacrol in a Ca^{2+}-dependent manner. Furthermore, this peptide inhibits
718 AITC-induced release of CGRP from mouse trachea and reduces hypersensitivity to
719 cold produced by ciguatoxin, and the mechanical hypersensitivity induced by
720 bradykinin or by the yeast cell wall polysaccharide zymosan (125). Along a similar
721 line, a peptidergic scorpion toxin (WaTx) activates TRPA1 by acting on same
722 intracellular site modified by reactive electrophiles (286, 475).

723 **6.2. NON-ELECTROPHILIC MODULATORS**

724 In addition to the huge number electrophilic activators, an ever-increasing list of
725 compounds that are unlikely to induce covalent modifications of the channel protein
726 can activate TRPA1 (119, 959).

727 Parabens, alkyl esters of p-hydroxybenzoate, are added to pharmaceuticals,
728 cosmetics and food products as antibacterial agents. Methyl-paraben (methyl p-
729 hydroxybenzoate) is an activator of TRPA1 and produces pain in mice that is blocked
730 with ruthenium red (258).

731 3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597) is a potent inhibitor of the
732 anandamide-degrading enzyme fatty acid amide hydrolase (FAAH). URB597, but not
733 two other FAAH inhibitors URB532 and Compound 7, activates human and rat
734 TRPA1. URB597 activates rat TRPA1 in inside-out patch-clamp experiments and
735 stimulates TRPA1-expressing rat dorsal root ganglion neurons that also respond to

AITC. In contrast, URB597 inhibits TRPM8 and is ineffective on TRPV1 and TRPV4 (591).

The super-cooling chemical agent icilin (AG-3-5) activates not only TRPM8 but also TRPA1. This compound triggers a rapid, long-lasting and dose-related hyperthermia in rats, which is attenuated by pretreatment with the nitric oxyde synthase (NOS) inhibitor N(G)-nitro-L-arginine methyl ester hydrochloride (L-NAME). Thus, TRPM8/TRPA1 mediated requires both NO production and NMDA receptor activation (211).

Surprisingly, also some frequently used therapeutic compounds such as Ca^{2+} antagonists have been identified as TRPA1 activators. Four 1,4-dihydropyridines (nifedipine, nimodipine, nicardipine and nitrendipine), and the structurally related L-type Ca^{2+} channel agonist BayK8644, activate TRPA1 (245). TRPA1 activation in perivascular nerves of resistance arteries produces vasodilation via the release of CGRP (76). However, it remains to be investigated whether the stimulatory action of 1,4-dihydropyridines on this channel has any relevance in the action of these compounds as antihypertensive drugs. A relatively simple experiment to be performed along this line is to test whether the vasodilation response of resistance arteries to 1,4-dihydropyridines is weaker in *Trpa1*-deficient than in wild type animals. Of note, it would be also interesting to determine the role of TRPM3, another sensory TRP channel that is activated by nifedipine (218) and whose activation in perivascular nerves induces dilation of resistance arteries (19).

Another surprise came with the identification of local anesthetics (LAs), which suppress cellular excitability by inhibiting voltage-gated Na^+ channels, as activators of TRPA1. Lidocaine activates TRPA1 in a concentration-dependent manner and its action is blocked by HC-030031. However, lidocaine can also act as an inhibitor of

761 TRPA1, an effect that is more obvious in rodent than in human TRPA1. This species-
762 specific difference is probably related to the transmembrane domains 5 and 6
763 forming the pore region (460). At high concentrations (30 mM) lidocaine induces Ca^{2+}
764 release from intracellular stores, an effect that may mediate TRPA1- and TRPV1-
765 independent CGRP release and cell death mouse DRG neurons (229). Lidocaine,
766 procaine and tetracaine induce glutamatergic spontaneous excitatory transmission in
767 substantia gelatinosa (SG) neurons (660, 661). Lidocaine dose-dependently and
768 reversibly increases the frequency, but not the amplitude of spontaneous excitatory
769 postsynaptic current (sEPSC) in SG neurons. This presynaptic enhancement is due
770 to activation of TRPA1 in nerve terminals presynaptic to SG neurons that increases
771 the spontaneous release of L-glutamate (660). Of note, TRPA1 is activated and
772 permeated by the lidocaine derivative QX-314, resulting in a concentration- and use-
773 dependent inhibition of cotransfected $Na_V1.7$ channels. These findings further
774 support the development of selective inhibition of action potential firing in nociceptive
775 neurons (779).

776 Importantly, non-electrophilic compounds may have electrophilic metabolites. For
777 instance, when acetaminophen (N-acetyl-p-aminophenol, APAP), the most common
778 antipyretic/analgesic medicine worldwide is overdosed, its metabolite, N-acetyl-p-
779 benzo-quinoneimine (NAPQI) causes liver damage. NAPQI is an electrophilic
780 molecule and stimulates TRPA1 causing airway neurogenic inflammation. These
781 inflammatory responses evoked by NAPQI and APAP can be abolished by TRPA1
782 antagonists (580).

783 NPPB (5-nitro-2-(3-phenylpropylamino)-benzoate), a classic Cl^- channel blocker,
784 potently activates human TRPA1. The action of NPPB suggests a possible close
785 interaction between S5 and N-terminal domains of the channel. As indicated by the

786 analysis of NPPB derivatives, NPPB activates TRPA1 through a structure-specific
787 mechanism (481).

788 Anesthetic agents generally induce a paradoxical activation and sensitization of
789 TRPA1. Propofol (2,6-diisopropylphenol) and etomidate are pungent and elicit
790 intense pain upon injection. The former compound is commonly used as intravenous
791 anesthetic (251). This compound was identified as TRPA1 agonist in a study that
792 also described the agonist effect of other alkyl phenols, such as thymol, 2-tert-butyl-
793 5-methylphenol and carvacrol (458), see below. The effects of propofol on sensory
794 neurons may contribute to peripheral sensitization to nociceptive stimuli in
795 traumatized tissue and may be therefore clinically relevant (936). TRPA1 activation
796 mediates the decrease in mean arterial pressure and dilation of murine coronary
797 microvessels induced by propofol, via a mechanism involving activation of NOS and
798 BK_{Ca} channels (742, 743) and restores the sensitivity of TRPV1 via NOS-dependent
799 activation of protein kinase C ε (PKCε) (744). Interestingly, the related compound
800 fospropofol does not produce pain upon injection and is unable to evoke depolarizing
801 currents in sensory neurons (650). Propofol-induced desensitization of TRPA1 was
802 proposed to mediate the inhibition of responses of wide dynamic range (WDR)
803 neurons to noxious heat (analgesia) and the reduction of AITC sensitization of WDR
804 neurons (antihyperalgesia), which agrees with clinically observed reduction in
805 postoperative pain in surgical patients anesthetized with propofol (799). It must be
806 noted, however, that TRPA1 is not the sole target of propofol in nociceptive neurons,
807 as it was reported that this compound may produce pain via TRPV1 activation and
808 voltage-gated Ca^{2+} channels downstream of γ-aminobutyric acid A (GABAA) receptor
809 activation (251, 599). Recent structure-function, molecular modeling and photoaffinity
810 labeling studies strongly suggest that propofol binds TRPA1 at a site located within

811 the S5-S6 pocket by forming H-bond and halogen-bond interactions with the amino
812 acid residues S876, M915, and M956 (820, 897).

813 General anesthetics (GAs) can activate peripheral nociceptive neurons in addition to
814 their known depressing action on the central nervous system. There are, however,
815 differences between the effects of these molecules, as for instance isoflurane and
816 desflurane are pungent and able to activate TRPA1, whereas sevoflurane and
817 halothane are not pungent and ineffective on this channel. Intravenous and inhalation
818 of pungent GAs at clinical concentrations excite sensory neurons and induce pain-
819 related responses and activation of tracheobronchial sensory nerves, triggering
820 CGRP release and neurogenic inflammation (233, 264, 386, 509, 563). Furthermore,
821 TRPA1-dependent neurogenic inflammation is greater in mice anesthetized with
822 pungent compared with non-pungent anesthetics. The pro-nociceptive effects of GAs
823 combined with surgical tissue damage can lead to a paradoxical increase in
824 postoperative pain and inflammation (386, 509).

825 A similar paradoxical effect is known for fenamate nonsteroidal anti-inflammatory
826 drugs. Several NSAIDs activate TRPA1, including flufenamic, niflumic, and
827 mefenamic acid, as well as flurbiprofen, ketoprofen, diclofenac, and indomethacin.
828 The response to fenamate agonists was blocked by TRPA1 antagonists. Fenamate
829 NSAIDs also potentiate the activation of TRPA1 by electrophilic compounds (334). It
830 should be noted that the NSAIDs diclofenac, ketorolac and xefocam suppress
831 thermal and mechanical hyperalgesia following TRPA1 activation, seemingly due to
832 channel inactivation or desensitization (603, 830, 831). Along the same line etodolac
833 activates via covalent modification of cysteine residues, and subsequently
834 desensitizes the channel (871).

TRPA1 activation was proposed to mediate the irritation caused by primary alcohols in skin, eye and nasal mucosa. Higher alcohols such as also activate TRPA1 with potency proportionally increasing with the carbon chain length. Interestingly, although these alcohols are not electrophilic, the residues C665 and H983 in the N terminus are required for their stimulatory action on TRPA1. Straight-chain secondary alcohols and primary and secondary alcohols activate hTRPA1 with a potency correlated with their octanol/water partition coefficients (416).

Human TRPA1 is activated in an irreversible manner by low micromolar concentrations of apomorphine, a non-narcotic derivative of morphine. At higher concentrations this compound produces an irreversible activation of the channel, resulting from a destabilization of the open state. TRPA1 activation may therefore be implicated in the ulceration and pain reactions at the injection site during treatment with apomorphine (719).

TRPA1 is activated by taurolidine, a compound clinically used as an antimicrobial or to exert newly recognized antineoplastic actions. This action results in release of CGRP from nociceptive nerve endings in isolated mouse trachea. The metabolite taurultam and its oxathiazine derivative had comparatively weaker effects (387).

Glibenclamide is an anti-diabetic drug that stimulates insulin release through the inhibition of K_{ATP} channels in pancreatic beta-cells. However, this compound may produce abdominal pain, gastrointestinal disturbances and nocturia. Glibenclamide activates human TRPA1 at concentrations similar to those acting on K_{ATP} channels *in vitro* and pharmacological and genetic evidence indicates that TRPA1 is implicated in the stimulatory effects of glibenclamide in mouse primary sensory neurons (55).

TRPA1 activation may account for the frequent adverse effects observed in patients treated with phenytoin. This compound activates a nonselective cationic current in

human gingival fibroblasts with typical features of TRPA1 channels, including inhibition by HC-030031 and reduction by shRNAs against hTRPA1. Phenytoin effects on these cells are strongly decreased the antioxidant vitamins ascorbic acid, folic acid, and alpha-tocopherol. Phenytoin induces accumulation of collagen in the extracellular matrix, but did not enhance cell proliferation (486).

6.3. ACTIVATION BY NATURAL COMPOUNDS

A significant number of sensory TRP channel activators appeared during evolution as chemical weapons produced by plants against herbivores. As recently reviewed (766), this fascinating subject has as notable examples the activation of TRPA1 by compounds produced by *Brassica* (mustard) and *Allium* (onions and garlic) plants, such as pungent isothiocyanates and allicin, respectively. Of note, other plants such as those of the genus *Cinnamonum* (cinnamon) produce TRPA1 agonists, but these compounds have more an antibacterial function than a repellent one. What these plants do have in common is that they have found a very special place in our culinary cultures and in the history of Mankind through their importance in commerce and therefore in the dominance over trade routes (592, 593, 878).

As already mentioned above, the spice wasabi (from *Wasabia japonica*) acquires pungency through its active compound AITC. The related compounds 6-(methylsulfinyl)hexyl isothiocyanate (6-MSITC) and 6-(methylthio)hexyl isothiocyanate (6-MTITC) have lower pungency, and are responsible for the fresh flavor of wasabi and act as TRPA1 specific electrophilic activators (837). The other most prominent TRPA1 agonist is cinnamaldehyde, the main constituent of cinnamon oil from *Cinnamonum verum* and other cinnamon species (61). This spice is widely used as a condiment and flavoring component in chocolate, many dessert recipes, such as apple pie, donuts, and in spicy candies, tea, hot cocoa, liqueurs, and even in

885 toothpastes. Given orally, it causes burning and tingling due to activation of TRPA1.
886 When applied topically on the skin, cinnamaldehyde produces spontaneous pain,
887 heat and mechanical hyperalgesia, cold hypoalgesia, neurogenic axon reflex
888 erythema, intensified warm sensations, reduced heat pain threshold, moderate itch,
889 flare, hyperknesis, alloknesis and increased skin blood flow and temperature (30, 53,
890 135, 322, 575, 630, 740). In the human tongue cinnamaldehyde enhances both heat-
891 and cold-induced pain (14, 685), increases the local temperature (353) and produces
892 burning sensation (301). When applied to human airways this compound produces
893 smart (stinging pain) (16), cough (365) and weak burning sensation (134). From the
894 first report on its action on TRPA1, cinnamaldehyde was described as more specific
895 than AITC (61). Hence, the responses to cinnamaldehyde *in vitro* and *in vivo* had
896 been widely taken as proof for functional expression of TRPA1 (889). However, it this
897 compound may have off-target effects, such as the described inhibitory effect on
898 voltage-gated Ca^{2+} channels in resistance arteries and concomitant vasodilation,
899 which could make an impact on nociception and local circulatory responses and
900 thermoregulation (26).

901 As TRPV2, TRPA1 is one of the newly described ionotropic cannabinoid receptors
902 (10). It is activated by the non-electrophilic ∆9-tetrahydrocannabinol (∆9-THC or
903 THC), the psychoactive compound in marijuana (*Cannabis sativa*) (357). This THC
904 effect does not require the presence of polyphosphates in the intracellular solution, in
905 contrast to that of AITC. This suggests for distinct mechanisms underlying the action
906 of these compounds (145). The cannabinoid receptor agonists WIN 55,212-2 and
907 AM1241 activate TRPA1 and thereby decrease the responses to capsaicin and
908 AITC. Moreover, TRPA1 is required for the inhibitory action of these compounds on
909 the nocifensive behavior elicited by capsaicin in mice (11). The activation of TRPA1
910 by fitocannabinoids was further confirmed in a study testing the action of cannabidiol,

911 THC, cannabidiol acid, THC acid, cannabichromene, and cannabigerol, which
912 produce intracellular Ca^{2+} responses comparable in amplitude to that evoked by
913 AITC (192). Synthetic analogues of phytocannabinoids were also reported to activate
914 the channel (129, 485, 666). TRPA1 mediates part of the inhibition of the ongoing
915 activity of ON and OFF neurons of the rostral ventromedial medulla in anaesthetized
916 rats induced by cannabidiol and cannabichromene, two non-psychoactive
917 cannabinoids. This effect is emulated by AITC. Cannabidiol and cannabichromene
918 also elevate endocannabinoid levels in the ventrolateral periaqueductal grey. These
919 findings open the possibility of using these compounds to target TRPA1 to produce
920 antinociceptive effects at the supraspinal level (498).

921 TRPA1 also mediates THC-induced CGRP release and vasorelaxant responses to
922 sensory nerve stimulation. Notably, morphine and its derivatives are commonly in
923 pain control but have proalgesic effects at high concentrations, as for instance the
924 induction of short-lasting painful sensations upon dermal application and CGRP
925 release via TRPA1 activation. Naloxone has analogous effects (254). Spinal TRPA1
926 also contributes to morphine antinociceptive tolerance via a positive feedback
927 mechanism that upregulates the H_2O_2-producing astroglial enzyme d-amino acid
928 oxidase (884). TRPA1 is a target of the farnesyl prenylogue of cannabigerol (CBG, 1)
929 found in the waxy fraction from the variety Carma of fiber hemp (*Cannabis sativa*).
930 This plant may contain additional prenylogous versions of medicinally relevant
931 cannabinoids, for which their biological profiles could be of potential therapeutic
932 interest (667). Of note, also cannabinoid receptor antagonists such as AM251 and
933 AM630 activate TRPA1, illustrating the importance of their off-target effects (647).

934 *Allium sativum* (garlic) produces the organo-sulfur pungent electrophile allicin, from
935 which other active derivatives are produced: diallyl sulfide, diallyl disulfide and diallyl
936 trisulfide. Allicin is a protective compound acting against endothelial cell dysfunction

937 in a rat model of right ventricle hypertrophy (786). TRPA1 activation by allicin (76,
938 492) could mediated this effect, if considering that TRPA1 is expressed in heart
939 (773). The allicin derivative ajoene, despite containing reactive electrophilic groups,
940 is unable to activate TRPA1, but enhances the activation by other electrophiles such
941 as AITC and allicin (925). It must be noted that allicin and its derivatives also activate
942 TRPV1, although with lower potency than for TRPA1 and with a lower efficacy
943 compared to capsaicin (412, 492). Nevertheless, this unspecificity should be taken
944 into account when extracting conclusions over the role of TRPA1 in visceral
945 sensitivity and motility from experiments based on the use of garlic (and allicin) as
946 agonist of this channel (257). Interestingly, allicin-induced activation of TRPV1 is
947 mediated by a single cysteine residue in the N-terminus of the channel, suggesting
948 for a similarity with the mechanisms of action operating in TRPA1 (706). TRPA1
949 activation was proposed to mediate the hyperthermic effects of the durian fruit (from
950 *Durius zibethinus Murr.*), which contain the allicin-related compound diethyl disulfide
951 (814). S-alkyl-S-alkenyldisulfides, a rare class of natural products contained in the
952 foul-smelling gum-resin of *Ferula assa-foetida L*. These compounds showed a
953 transthiolation capacity and potently activate TRPA1, suggesting this channel as the
954 culprit of potential beneficial effects associated to the use of asafoetida as a spice
955 and medicine (739).

956 Over 80 terpenes are produced by plants, fungi and animals, serving as deterrents of
957 foragers and predators. These compounds feature reactive unsaturated dialdehyde
958 moieties. The noxious fungal sesquiterpene isovelleral activates TRPA1, thereby
959 exciting sensory neurons and eliciting nocifensive responses in mice. Another
960 drimane sesquiterpene, polygodial (from *Polygonum hydropiper* leaves), also
961 activates TRPA1 (240). Miogadial, miogatrial and polygodial, all with an unsaturated
962 1,4-dialdehyde moiety, are stronger TRPA1 agonists than AITC (346). Interestingly,

963 although isovelleral contains a β-unsaturated dicarbonyl moiety potentially capable of
964 forming Michael adducts, it activates TRPA1 with mutated reactive cysteines,
965 suggesting that dialdehyde sesquiterpenes may act through a mechanism distinct to
966 that operating in other electrophiles (68). Interestingly, non-electrophilic
967 sesquiterpene such as α-, β-eudesmol and γ-eudesmol also activate TRPA1 (622).
968 These compounds are found in hop essential oil and are thought to confer a spicy
969 note to beer. The activation of hTRPA1 by β-eudesmol requires the residues T813,
970 Y840 and S873.

971 Another prominent TRPA1 agonist is menthol (from *Mentha piperita*). This compound
972 was first described to have no effect on TRPA1 (777) or to be an inhibitor of TRPA1
973 responses triggered by cold or cinnamaldehyde (493). However, it was later shown to
974 stimulate basal currents in the low micromolar range and to have an inhibitory action
975 beyond a few hundred μM (371) (Figure 4). Washout of inhibitory concentrations of
976 menthol leads to a significant rebound of TRPA1 activity, which is consistent with a
977 very fast un-blocking event. The agonist effect of menthol is due to a shift of the
978 voltage dependence of channel activation to negative potentials. The inhibitory effect
979 relates to an increased probability of null traces and the appearance of fast flickering
980 of single-channel currents, raising the possibility that menthol acts as an open pore
981 blocker.

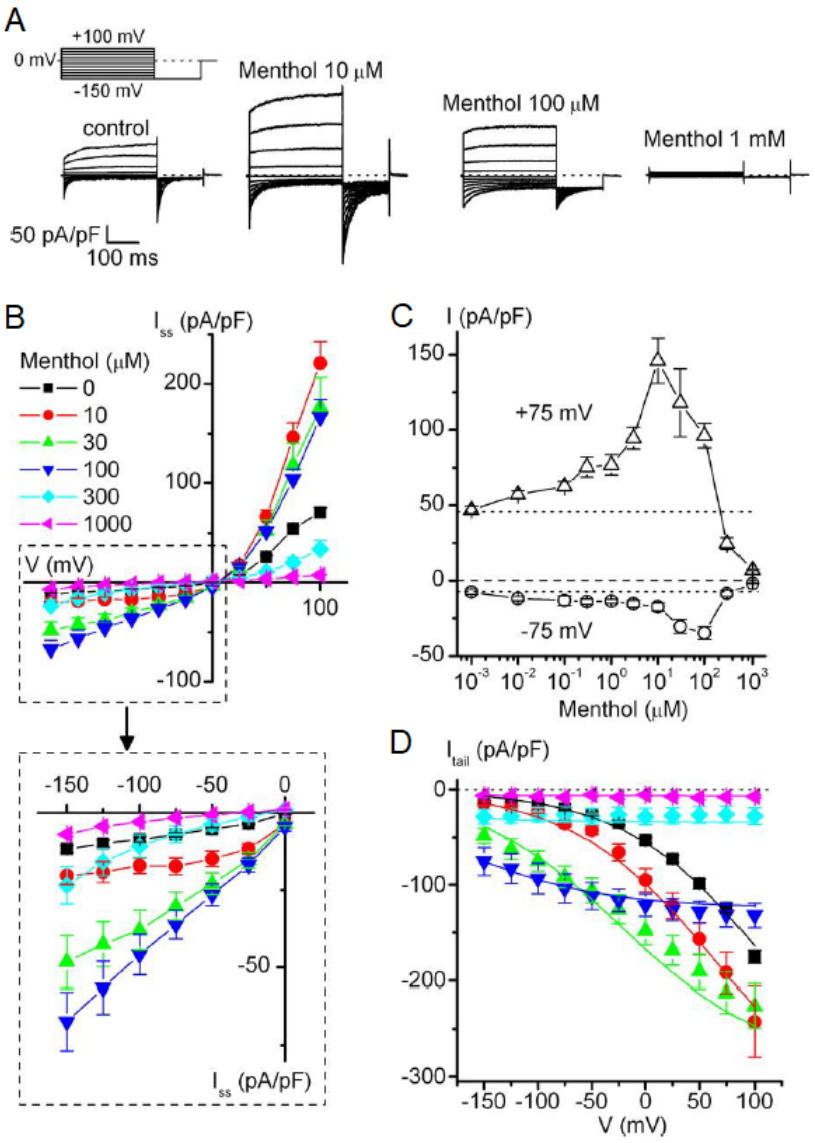

Figure 4: Bimodal action of menthol on the gating properties of TRPA1. (A) Whole-cell TRPA1 currents in response to the indicated voltage protocol under control conditions and during application of 10 and 100 μM and 1 mM menthol. **(B)** I–V relationships obtained at the end of the 300 ms voltage steps shown in **(A)**. Dose–response relationships of menthol on TRPA1 at the indicated voltages **(C)**. Dotted lines indicate constitutive currents before menthol application. **(D)** Tail currents at −150 mV in control and during application of the different concentrations of menthol. Modified with permission from Karashima *et al*., (371).

982 Notably, several modulators of TRPA1 act in such a bimodal fashion. Importantly,

983 hTRPA1 is activated by menthol but not inhibited by it, whereas TRPA1 from non-
984 mammalian species are reported to be insensitive to this compound. Structure-
985 function studies on mouse, human and *Drosophila* TRPA1 isoforms led to the
986 identification of the pore region TM5 and TM6 as the critical domain determining
987 whether menthol can act as an inhibitor and of specific residues in TM5 (S873 and
988 T874) required for menthol-induced activation. It remains, however, unclear whether
989 these structures are actually involved in binding or channel gating (908).
990 Nevertheless, human TRPA1 emerged as a highly sensitive menthol receptor that
991 very likely contributes to the diverse psychophysical sensations after topical
992 application such as warmth, burning, irritation and pain (168, 575). These findings
993 were crucial to re-interpret conflicting data on the sensory roles of TRPM8 and
994 TRPA1 that raised from the wrong assumption that menthol was specific for the
995 former channel (860). They help expaining, for instance, the existance of menthol-
996 responsive neurons in *Trpm8*-deficient animals (170, 207) and the role of TRPA1 as
997 cold sensor in visceral nociceptive neurons (244).

998 TRPV1, and to a lower extent TRPA1, are molecular targets of hydroxy-α-sanshool,
999 from Szechuan peppers (*Zanthoxylum piperitum*), an effect that was proposed to
1000 underlie some of the irritant, cooling, tingling and paresthetic sensations these spices
1001 produce in the tongue (420). This view was, however, challenged by a subsequent
1002 study reporting that hydroxy-α-sanshool has minor effects of these channels and only
1003 at high concentrations, but on the other hand inhibit the 2-pore potassium channels
1004 KCNK3, KCNK9 and KCNK18 (77). The later effect was then proposed to mediate
1005 Szechuan peppercorns sensory properties referred as “tingling and numbing”, “mild
1006 electric shock” or “pins and needles” through the combination of the targeting of
1007 capsaicin-sensitive nociceptors and mechanosensitive neurons. This view was
1008 further supported by psychophysical experiments showing that application of

isobutylalkenyl amide induce multifaceted and dynamic sensations in human tongue (15), as well as complex aversive responses in the rat (398). Nevertheless, these initial findings served as starting point for the development of new hydroxy-α-sanshool-derived alkylamides manifesting higher selectivity for TRPA1. The cis C6 double bond in the polyenic chain of a-SOH is critical for TRPA1 activation and no structural specificity is required for TRPV1 activation (521). Alkylamides and 6-shogaol act on TRPA1 by covalent bonding, whereas none of these compounds activate TRPV1 through such interactions (391, 681).

Thymol (2-isopropyl-5-methylphenol) is a natural monoterpene phenol derivative of cymene, isomeric with carvacrol. This compound is enriched in the oil of the thyme plant (*Thymus vulgaris*) and has a pleasant aromatic sharp odor as well as a pungent flavor. Thymol potently activates TRPA1 and desensitizes it to further exposure to thymol or AITC. Other phenols such as 2-tert-butyl-5-methylphenol, 2,6-diisopropylphenol (propofol) and carvacrol also activate hTRPA1. Phenols with more bulky carbon substitutions and hydrophobicity are more potent activators (458). TRPA1 is also activated by the related monoterpenoids p-cymene-3-carboxylic acid, and 3-amino-p-cymene (632) and limonene (361). Thymol was later found to have in fact a bimodal action on TRPA1 (371) and to share the same required structural domains for channel activation with menthol (908). This compound increases the spontaneous release of L-glutamate onto spinal substantia gelatinosa neurons, an effect that is antagonized by HC-030031 (917). TRPA1 was proposed to mediate the pungency of carvacrol, a compound found in the spice oregano, derived from *Origanum vulgare*, which is used in the kitchen for the aromatic, warm and slightly bitter taste of its leaves (458, 915). Intraepidermal injection of carvacrol in humans produces concentration-dependent pain sensations that are reduced by the TRPA1 antagonist A-967079 (722). TRPA1 is activated by eugenol (164) and gingerol-

related compounds (552). The former is a phenylpropene found in cloves (*Syzygium aromaticum*) and it is used in perfumes, flavorings and essential oils and in medicine as a local antiseptic and anesthetic. Gingerol is a phenol-derived compound extracted from the essential oils of clove and fresh ginger (*Zingiber officinale*). The related compounds L-carveol, trans-p-methoxycinnamaldehyde, methyl eugenol, 4-allylanisole, and p-anisaldehyde, contained in the stem and leaves of *Agastache rugosa* (of the *Labiatae* family), selectively activate hTRPA1 over hTRPV1 (546). Of note, thymol, carvacrol and eugenol are well-known agonists and sensitizers of TRPV3 in skin keratinocytes (915). The ability of these chemicals to increase the avoidance of warmer temperatures supported a role for TRPV3 in warmth detection (399). However, the recent reports on heat-induced activation of mammalian TRPA1 and on its implication in heat sensing (see below) indicate that this channel may also underlie the modulation of warmth sensing by such compounds.

Another example for a species dependent activation of TRPA1 is caffeine (from *Coffea arabica*). This compound activates mouse TRPA1 but suppresses human TRPA1. The region between the amino acid residues 231 and 287, in the distal N-terminal region of mTRPA1, is critical for this effect. The point mutation M268P changed the effect of caffeine from activation to suppression in mTRPA1 (565, 566).

Also (-)-nicotine *(*from *Nicotiana tabacum*) acts as a bimodal TRPA1 modulator. TRPA1 activation may therefore mediate part of the irritation induced by topical application of nicotine as used in nicotine replacement therapies on the mucosa and skin (801) or by the use of electronic cigarettes (165). It is thus thought that TRPA1 block may facilitate the development of smoking cessation therapies with less adverse effects. The nicotine congenitor, anabasine (from *Nicotiana glaucum*), acts in the same way as nicotine (801). (+)-nicotine was later reported to activate hTRPA1 (718).

1'-acetoxychavicol acetate is the major pungent component in galangal (galanga, blue ginger), a rhizome of plants of the genus *Alpinia officinarum* originated from Indonesia that has medicinal and culinary uses. This compound activates TRPA1 more potently than AITC, but not TRPV1 (576). It remains unclear, however, whether any of the properties of galangal, which include antioxidative, antigastric ulcer and antitumor effects, and potentially enhanced thermogenesis, are related to TRPA1 activation.

The spiciness of black pepper (*Piper nigrum* from the family *Piperaceae*) is due to the chemical piperine. This, and the related compounds isopiperine, isochavicine, piperanine, piperolein A, piperolein B, and N-isobutyl-(2E,4E)-tetradeca-2,4-diamide strongly activate TRPA1 (628). This effect, and the complementary action on TRPV1, was proposed to mediate the beneficial effects of piperine through stimulation of oropharyngeal sensory neurons that may serve as novel therapeutic strategy against dysphagia (25, 688) and piperine-induced contraction in guinea-pig ileum, urinary bladder and trachea (86).

Curcumin is an active principle of the turmeric root (*Curcuma longa*) of the ginger family (*Zingiberaceae*). This compound is used in many cuisines as “yellow curry” and can form Michael adducts. Curcumin activates human TRPA1 but has no effect on TRPM8 or TRPV1 (453). Within a series of 33 synthetic curcumin analogues, 20 acted as TRPA1 modulators, 6 of the 1,3-dicarbonyl and acyclic series behaved as TRPM8 antagonists and only a few were able to either inhibit or activate TRPV1 (573).

Oleocanthal is a natural phenolic compound known to mediate the pungency of extra-virgin olive oil (166). Human TRPA1 is selectively activated by oleocanthal and is required for the stimulation of rodent trigeminal neurons. Results from

immunohystochemical localization of TRPA1 and psychophysical experiments in humans point to a restricted functional expression of this channel in the pharynx (657). Similar findings obtained with the over-the-counter analgesic ibuprofen agree with shared chemesthetic qualities with olive oil (tickle) (93), and with the fact that the former activates human TRPA1, though not directly, via its electrophilic metabolite ibuprofen-acyl glucuronide. Interestingly, ibuprofen-acyl glucuronide impares subsequent channel activation by AITC, suggesting that it may mediate part of the analgesic and anti-inflammatory activities of ibuprofen (186).

The 'headache tree', California bay laurel (*Umbellularia californica*) is celebre for causing severe headache crises upon inhalation of its vapors. The monoterpene ketone umbellulone is the major volatile constituent of the leaves of this plant, and has irritating properties, probably due to its ability to rapidly bind to thiol groups. Umbellulone has a bimodal action on TRPA1, stimulating at low concentrations and inhibiting at high concentrations. The former effect is reduced, but not completely abolished in the TRPA1 mutant C622S. Analogues of umbellulone with removed Michael acceptor properties have reduced stimulatory effects but increased inhibitory potency. Several umbellulone derivatives induce weak TRPM8 activation (943). Multiple sensory responses to umbellulone, such as stimulation of nociceptive neurons, release of CGRP and nocifensive behavioral responses are abrogated in *Trpa1*-deficient mice. Systemic administrations of TRPA1 and CGRP receptor antagonists inhibit the increased rat meningeal blood flow induced by intranasal application or intravenous injection of umbellulone. These data suggests that other noxious agents such chlorine, cigarette smoke and formaldehyde produce headaches via TRPA1 activation in trigeminal nerves (583), and illustrate how the study of the mechanisms underlying the action of herbal compounds may help in the understanding of pain (278).

Ligustilide is the major contributor of the aroma of celery (from *Apium graveolens*, *Levisticum officinale*, from popular medicinal plants used in traditional Chinese medicine such as *Angelica sinensis*, *Ligusticum chuanxiong*, and North American traditional Medicine from *Ligusticum portieri*). This electrophilic volatile dihydrophthalide is a potent activator and modest inhibitor of TRPA1. Similar to what was found for umbellulone, the aromatization of ligustilide to dehydroligustilide reduces the stimulatory effect and enhances the inhibitory effect on TRPA1. It was proposed that TRPA1 contributes to the flavor and pharmacological actions of celery and other important plants from the Chinese and native American traditional medicines (944). Later on, a series of ligustilide analogues (3-ylidenephthalides) were synthetized and found to be strong modulators of TRPA1 and TRPM8 (633).

Capsiate, produced by “sweet” *Capsicum annuun L.*, is a non-pungent cultivar of red pepper, and, like capsaicin, it is considered to boost metabolism by activating the sympathetic nervous system and suppressing inflammation. TRPA1 is activated by the capsinoids capsiate, dihydrocapsiate and nordihydrocapsiate, although with potency lower than that for TRPV1 activation (738). Interestingly, benzylamide-to-tetrazole substitutions in the vanilloids olvanil, rinvanil, and phenylacetylrinvanil resulted in non-electrophilic compounds with the ability to activate TRPA1 (199).

Artepillin C, a prenylated derivative of cinnamic acid, is the main pungent ingredient in Brazilian green propolis, a popular health supplement used for its various biological properties. Artepillin C activates TRPA1 more potently than AITC. Other cinnamic acid derivatives such as baccharin and drupanin also activate TRPA1, whereas p-coumaric acid has no effect (305).

The hydroalcoholic extracts of the first leaves of *Kalopanax pictus Nakai* (*Araliaceae*) activate human TRPA1 and TRPV1 channels (758). Out of six commercially available

compounds (methyl syringate, coniferyl alcohol, protocatechuic acid, hederacoside C, alpha-hederin, and eleutheroside B) found in *K. pictus*, methyl syringate was shown to be ineffective on hTRPV1 and to activate hTRPA1, although with lower potency than AITC (757). It was latter shown that methyl syringate inhibits the hypoxic induction of cyclooxygenase-2 (COX-2) expression and invasion through TRPA1 activation in adenocarcinomic human alveolar basal epithelial A549 cells, suggesting that this compound may be of value to abrogate hypoxia-induced inflammation (641).

Parthenolide is a major constituent feverfew (*Tanacetum parthenium*), a traditional medicinal herb that has been used for centuries to treat pain and headaches. This compound stimulates recombinant and native rat and mouse TRPA1 channels, behaving as a partial agonist. Interestingly, parthenolide desensitizes TRPA1 causing unresponsive of peptidergic TRPA1-expressing nerve terminals, an effect that may contribute to the antimigraine effect of parthenolide (506).

Furylketones derivatives are another class of chemical structures active with high potency on TRPA1 (73). These compounds relate to the naturally-occurring perillaldehyde and perillaketone extracted from *Perilla frutescens*, a plant widely used in Asian cuisine. Both are activators of TRPA1, which may explain the chemesthetic properties of this plant. Perilla leafs (zisuye) cause warm sensations and are widely used in traditional Chinese medicine and against stomach dysfunction (72).

TRPA1 and TRPV1 are activated by theasinensins A and D, two auto-oxydation products of epigallocatechin gallate, a polyphenol contained in green tea. This effect might contribute to the astringent sensation produced by this compound in the tongue (440, 441).

Despite of their several beneficial effects, such as in the treatment of skin disorders and cancer, retinoids produce irritation side effects. The retinoid receptor RARβ antagonist LE135 stimulates DRG neurons and produce pain-related behaviors via activation of TRPA1 and TRPV1. A point mutation K170R abrogates the stimulatory effect on TRPA1 (927).

Anethole, present in anise, fennel and liquorice, activates native mouse TRPA1 in DRG and TG neurons and recombinant human TRPA1 in HEK293 cells. The mechanism of activation by this compound seems to require the same residues involved in stimulation by menthol, S873 and T874 in TM5. Anethole desensitizes and for unknown reasons fails to produce nocifensive behaviors in mice (518).

Other reported TRPA1 agonists of natural origin are leucettamols, bifunctionalized sphingoid-like compounds from a marine sponge *Leucetta* sp. (157), C_{14} polyacetylenes from the plant *Echinophora platyloba* (*Apiaceae*) (158), compounds from the Himalayan ritual medicinal plant *Waldheimia glabra* (277),

6.4. Ca^{2+}-DEPENDENT MODULATION

The initial studies on functional properties of TRPA1 were already consistent with a modulation of channel activation by intracellular Ca^{2+} (357). It was shown that human TRPA1 mediated a ruthenium red-sensitive large and rapid increase in intracellular Ca^{2+} concentration in response to carbachol in HEK293 cells co-expressing this channel and M1 muscarinic acetylcholine receptors (357) and in response to thapsigargin in TRPA1-expressing HEK293 (357), Chinese hamster ovary cells and sensory neurons (649). Furthermore, although Ca^{2+} was shown not to be critical for channel activation, its presence in the extracellular solution enhanced the responses

1185 to AITC and THC (357). The later results were further supported and extended to
1186 establish that Ca^{2+} also induces mouse TRPA1 current inactivation that is relieved by
1187 membrane depolarization (564). Taken together, these findings led to a model
1188 whereby extracellular Ca^{2+} permeating through the channel induce potentiation and
1189 inactivation by binding to a site within or very close to the pore (564).

1190 This model was confirmed by the observation of TRPA1 activation by increase in
1191 intracellular Ca^{2+} concentration through the patch pipette (213, 958) or via UV
1192 triggered uncaging (876) in whole-cell recordings and by applying Ca^{2+} directly on the
1193 cytoplasmic side of the membrane in the inside-out configuration (213, 876, 958) .
1194 Furthermore, the characterization of the rat TRPA1 pore mutant D918A in which Ca^{2+}
1195 permeability is greatly reduced resulted in no potentiation nor inactivation by
1196 extracellular Ca^{2+} (876). The activation and inactivation processes display distinct
1197 Ca^{2+} sensitivities (787, 876) and rat TRPA1 currents are inactivated by Mg^{2+}, Ba^{2+}
1198 and Ca^{2+}, but potentiated only by Ba^{2+} and Ca^{2+} (876), all of which suggests that both
1199 processes are independent (596, 876).

1200 The molecular mechanisms underlying Ca^{2+}-dependent modulation of TRPA1 have
1201 been a matter of debate and require further study, especially in native conditions
1202 (596, 876). It was initially proposed that Ca^{2+} activates TRPA1 by binding directly to a
1203 putative N-terminal EF-hand (213, 958), but later work reported that mutations in this
1204 region do not interfere with neither Ca^{2+}-dependent processes (596, 876). Later
1205 structure-function analyses of human TRPA1 revealed a functional similarity with the
1206 rat isoform and the implication of a distal C-terminal region of the human TRPA1 in
1207 Ca^{2+}- and voltage-dependent gating (787). Interestingly, the Ca^{2+}-dependent
1208 inactivation of both channels is faster at negative potentials (787, 876), suggesting
1209 that the inactivation is accompanied by a shift of the voltage dependence of channel
1210 activation to more positive potentials. The deletion of the last 26 amino acid residues

of the C-terminus of human TRPA1 results in a non-functional protein, whereas deletion of the last 20 has no significant effects on Ca^{2+}-induced potentiation, but decreases the rate of Ca^{2+}-dependent inactivation, again another argument for potentiation-inactivation uncoupling (787). Single mutations in a negatively charged amino acid stretch located in a preceding C-terminal region result in channels with various and intriguing properties, including shift of the voltage dependence of channel activation to less positive potentials (E1077A and E1077K) and delayed Ca^{2+}-induced potentiation in the mutants E1073A, D1080A, D1081A and D1082A. The possibility of this region to bind Ca^{2+} was supported by molecular dynamics simulations performed on a structural model of this stretch built from a Ca^{2+}-binding motif found in the human Ca^{2+}- and voltage-activated BK channel (hSlo1). Taken together, these findings indicate that the conserved acidic motif in the C-terminus of TRPA1 is directly implicated in the regulation of this channel by Ca^{2+} (787). However, it seems clear that much further work is necessary to determine the precise role of this region in the interplay of the effects of chemical agonists, membrane potential and permeating Ca^{2+} in the gating mechanisms of TRPA1. Intriguingly, another study reported direct interaction of TRPA1 and calmodulin that were only observed in the presence of Ca^{2+} (or Ba^{2+}, but not Mg^{2+}) (304). This interaction was shown to be mediated by the C-lobe of calmodulin and a non-canonical calmodulin-binding domain formed by 17 amino acid residues in the C-terminus of TRPA1 and to be required for Ca^{2+}-dependent potentiation and inactivation of the channel.

Ca^{2+} is thus one of the most important endogenous modulators of TRPA1, producing activation secondary to signaling events leading to increase in cytosolic Ca^{2+} levels, amplification of initial activation by other stimuli of the channel, rapid termination of channel activation via the inactivation process, and refractoriness to subsequent channel stimulation. Furthermore, TRPA1 modulation by Ca^{2+} is a key element of the

interaction of this channel with TRPV1 (332, 649) (see below for more details: MODULATION BY PROTEIN – PROTEIN INTERACTIONS). It is also worth noting that the Painless channel from *Drosophila* and the TRPA1 channels from the green anole lizard, chicken and rat snake are also modulated by Ca^{2+} (435, 436, 753).

6.5. MODULATION BY METALS

The gating promiscuity of TRPA1 is further illustrated by the stimulatory effects of several metals, such as Zn^{2+}, Cd^{2+} and Cu^{2+}. Zinc, an essential biological trace element known to modulate the function of multiple proteins (902), activates mouse and human TRPA1. A series of permeability and structure-function experiments yielded as most plausible mechanism of Zn^{2+}-induced activation one in which Zn^{2+} permeating through the pore interacts with cysteine residues in the N- and C-termini and a histidine residue the C-terminus (67, 333). The EC_{50} for activation of hTRPA1 by intracellular Zn^{2+} in inside-out patches is around 50 nM (333). This value was shown to be even lower for mouse TRPA1 heterologously expressed in CHO cells (7.5 nM) in a study that also reported that this channel and its human isoform can be activated by extracellular application of the Cu^{2+}/Zn^{2+} ionophores clioquinol (an antifungal and amoebicidal drug) and zinc pyrithione (34). The *in vivo* relevance for these findings is supported by the findings that *Trpa1* KO mice display reduced nocifensive responses to hind paw injections of zinc acetate (333) and do not show the clioquinol-induced reduction in the threshold to paw pressure or the shorted withdrawal latency to cold (10 °C) (34), which were otherwise clearly observed in wild type animals. Furthermore, intratracheal instillation of $ZnCl_2$ reduces the respiratory rate in mice, an effect that is absent in *Trpa1* KO animals (289). It was suggested that activation of TRPA1 by Zn^{2+} induces secretion of cholecystokinin and gastric emptying in rats (568). On the other hand, TRPA1 is not involved in TRPV1 inhibition by intracellular Zn^{2+} (489), nor in Zn^{2+}-induced toxicity in HEK cells, or A549

(adenocarcinomic human alveolar basal epithelial) cells endogenously expressing this channel (770).

Zn^{2+}, Cd^{2+} and Cu^{2+} stimulate mouse pulmonary sensory neurons via a TRPA1-dependent mechanism (289). Cd^{2+} activates human (333, 531) and mouse (531) TRPA1 channels. As for Zn^{2+}, two cysteine residues and one histidine residue are implicated in Cd^{2+}-induced activation, this ion permeates the channel and TRPA1 is required for pain behaviors induced by intraplantar Cd^{2+} injection in mice (531).

6.6. MODULATION BY PH

Intracellular alkalosis activates mouse (259) and human (208) TRPA1 channels and these effects were proposed to mediate the channel stimulation by extracellular NH_4Cl. The mouse isoform is activated from a concentration of extracellular NH_4Cl of 1 mM, with an EC_{50} of 9.2 mM. Increasing the intracellular pH to 8.0 enhances channel opening in inside-out patches of HEK293 cells expressing mouse TRPA1 (259). The human and rat channel isoforms are less sensitive to alkalosis than the mouse one: hTRPA1 is activated only at pH 9.5 (208), whereas rTRPA1 is not activated even such high pH (875). These differences may be also due to the use of distinct experimental conditions. Human TRPA1 is less sensitive than rat TRPV1, which was activated at pH 7.8 (208).

An implication of N-terminal cysteine residues was consistently proposed to underlie the mechanism of alkalosis-induced activation. Intracellular alkalinization does not activate the double mutant C422S/C622 of mouse TRPA1 (259), whereas two human TRPA1 mutants show either complete (C621S) or partial (C665S) ablation of activation by NH_4Cl (208). Although it was proposed that a high intracellular pH enhances the action of endogenous electrophilic agonists that are retained in cell-

free membrane patches (259), the mechanisms whereby the proposed modifications of cysteine residues induce channel activation remain unknown.

The assessment of the effects of NH_4Cl on sensory neurons has yielded inconsistent results. On one hand, the increase in currents observed in a fraction of wild type mouse DRG neurons stimulated by 10 mM NH_4Cl was otherwise not observed in cells isolated from *Trpa1* KO animals (259). On the other hand, another study reported that the fraction of mouse DRG neurons responding in intracellular Ca^{2+} imaging experiments to 50 mM NH_4Cl was not different for cells isolated from wild type, *Trpv1* KO and *Trpa1* KO animals, but there was more than 50% reduction of the fraction of responsive neurons in *Trpa1* KO cells treated with the TRPV1 inhibitor BCTC (208). Altogether, and not considering the obvious differences in experimental design, it seems that TRPA1 may be required for the responses of mouse DRG neurons to NH_4Cl in the lower range of concentration.

The results of testing the implication of TRPA1 in nociceptive responses triggered by intraplantar injection of NH_4Cl in mice also gave apparently contrasting results. It was on one side shown that injection of 20 µl of 100 mM NH_4Cl induces clear nocifensive behaviors in wild type mice, being this responses virtually absent in *Trpa1* KO animals (259). On the other hand, injection of 10 µl of 375 mM NH_4Cl was reported to be the lowest concentration inducing robust nocifensive responses in wild type mice. Furthermore, these responses were not significantly different in *Trpv1* KO, *Trpa1* KO and *Trpa1* KO mice treated with BCTC or another TRPV1 antagonist AMG 9810 (208). Thus, it was proposed that other receptors may determine the pain induced by NH_4Cl. Taken together, it is clear that much more detailed evaluation of these responses needs to be made to determine what the precise contribution of TRPA1 to NH_4Cl-induced neuronal and behavioral responses really is.

Later *in vitro* studies concluded that rat TRPA1 is activated by intracellular acidification and that is implicated in the responses of trigeminal sensory neurons to CO_2 (875) and weak acids such as acetic, propionic, formic, and lactic acids (874). Such findings support the idea that noxious TRPA1 agonists activate the channel via specific chemical properties, such as electrophylicity, oxidative power and in this case, the ability to acidify the intracellular milieu, rather than through specific chemical structures (268).

The effects of extracellular protons represent another paradigm of interspecies differences regarding the modulation of TRPA1. Indeed, extracellular acidosis activates and sensitizes human TRPA1, whereas this stimulus is ineffective on the rhesus monkey isoform (184) and inhibits rat TRPA1 (875). Species differences in activation of TRPA1 by extracellular protons seem to result from differences in the amino acid sequence in the S5 and S6 transmembrane segments. Protons seem to interact with an extracellular site of human TRPA1 and not through modifications of N-terminal cysteine residues (184). These findings led to the proposal of TRPA1 as a sensor for extracellular acidosis in human sensory neurons, but a subsequent study revealed that the experimental TRPA1 antagonist A-967079 does not reduce the reported pain to a continuous intraepidermal injection of a pH 4.3 solution (722). Although it was concluded the TRPA1 can be excluded as a critical player in human acidosis-induced pain, the possibility that A-967079 does not inhibit the activation of human TRPA1 by extracellular protons has not been discarded.

6.7. ACTIVATION BY COLD AND HEAT

TRPA1 was initially described as a cold-activated channel (61, 777) (Figure 5), but this view was immediately controversial (75), and already discussed in multiple occasions (141, 446, 450-452, 512, 775, 776, 855, 861, 891, 959).

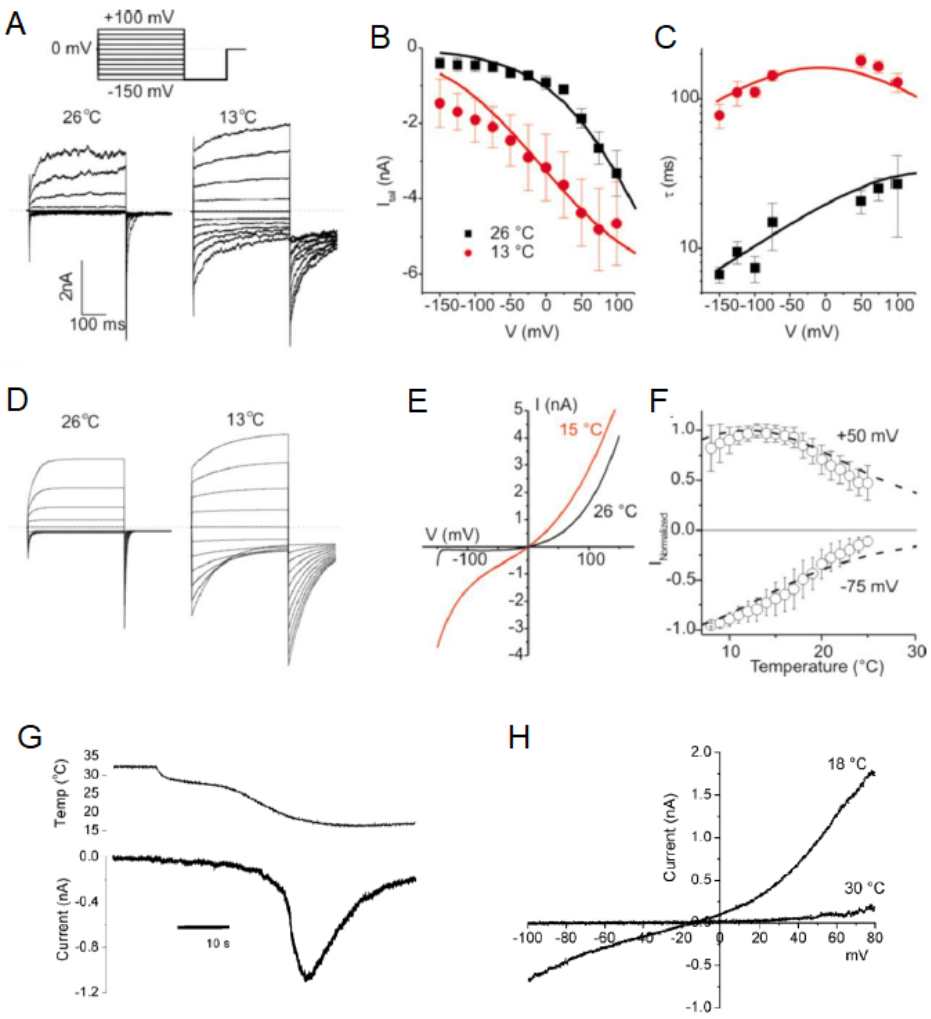

Figure 5: Activation of TRPA1 by cold. (A) Effects of cooling on the voltage-dependent gating and kinetics of TRPA1. Whole-cell currents in Ca^{2+}-free intracellular and extracellular solutions in response to the indicated voltage step protocol applied at 26 °C and 13 °C. **(B)** Average peak inward tail currents at −150 mV at 26 °C and 13 °C. **(C)** Average time constants obtained from monoexponential fits to the time course of current relaxation at different voltages and temperatures. **(D)** Model predictions of TRPA1 currents at 26 °C and 13 °C in response to the voltage step protocol in **(A)**. **(E)** Model predictions of TRPA1 currents during 400-ms voltage ramps. **(F)** Average TRPA1 currents at different temperatures and at −75 and +50 mV, normalized to the maximal current in the tested temperature range. **(G)** Representative traces of inward current evoked by cooling in TRPA1-expressing CHO cells. **(H)** I-V relationship of TRPA1 at 30 °C and 18 °C. Modified with permission from Karashima *et al*., (375) and Story *et al*., (777).

One of the key points in the discussion about the cold sensitivity of TRPA1 is whether TRPA1 channels have intrinsic sensitivity to cold. When trying to find an answer for this question, it is even difficult to define what “to have intrinsic sensitivity” really is, because to understand the function of an ion channel in a biologically relevant context, this protein should not be separated from its native membrane environment, its endogenous regulators, or its interaction partners. A recent study showed that human TRPA1 inserted in an artificial lipid bilayer is cold-sensitive, with and without the N-terminal ankyrin repeat domains (549). It was concluded that this channel is therefore “intrinsically sensitive” to cold. However, one may suggest that perhaps human TRPA1 is cold-activated when it is expressed in artificial membranes, but actually not so in its native environment. Thus, this may be an endless discussion. Nevertheless, there is fairly convincing evidence for activation of certain TRPA1 isoforms by cold in relatively intact cellular conditions. For instance, cold-induced activation of mouse TRPA1 was shown to occur in a Ca^{2+}-independent manner and at the single-channel level in cell-attached and in inside-out patches (375, 713). Thus, it could be argued that this phenomenon is at least membrane-delimited and does not require diffusible mediators. Nevertheless, the effects of positive and negative feedback loops due to Ca^{2+}-dependent activation and desensitization on the response to cold cannot be neglected in intact cells (876, 958).

Recent studies indicate that the thermal modulation of TRPA1 is even more complex. Purified human TRPA1 expressed in artificial membranes is activated by both cold and heat, with minimal open probability around 22 °C. Interestingly, the activation by cold and heat is modulated by redox modification. Reducing agents decrease the responses to heat and cold, whereas H_2O_2 produce a response at 22 °C, decrease the response to heating to 30 °C and increase that to cooling to 15 °C. Furthermore, the redox status and channel agonists modulates the responses of human TRPA1 in

HEK293 cells and warming to 36 °C under oxidative stress triggers a robust TRPA1-dependent CGRP release from mouse trachea (548). These results contrast with the conclusion of a previous report that warm temperatures suppress rat and human TRPA1 activity (873). Intriguingly, warming accelerates the activation of the channel induced by AITC, suggesting that heating favors the chemically-induced activation as previously suggested (375), but a concomitant more dramatic increase in the rate of channel desensitization may result in lower maximal responses to the agonist. Taken together, these findings highlight the complexity of the thermal modulation of TRPA1, demonstrate that environmental factors strongly shape the responses of this channel to thermal stimuli, and more importantly, clearly illustrate that much further research is required to fully understand the basic properties of TRPA1. Interestingly, TRPA1 may play a role in heat nociception (320, 847), possibly by contributing to the definition of the threshold for heat-induced responses in nociceptors (320). The mechanism underlying this role may be related to the ability of the channel to sense oxidative stress produced by heat (847).

We find several factors that may help to reconcile the different views on the role of TRPA1 in cold sensing. First, it seems clear that there are interspecies differences regarding the mammalian TRPA1 sensitivity to cold (151, 451). Second, the experimental conditions may vary significantly across different studies. For instance, HEK293 cells have reduced tolerance to transient transfection with mouse TRPA1, displaying intracellular Ca^{2+} overload, probably due to constitutive channel activation, and consequently less ability to further respond to cold and any other stimulus (375). There is also no homogeneity in the age of animals used to isolate sensory neurons (75, 319, 357, 375) and different laboratory may produce primary cultures of these cells in relatively different ways, using or not growth factors and complements, which could make a strong impact of the function of this heavily modulated channel (57,

548). Third, the experimental protocols may not always be optimal for detecting cold-induced activation of TRPA1. For instance, if cold is applied during stimulation with a strong agonist such as AITC, the open probability of the channel is not expected to increase much further and only the negative effect of cold on the open channel conductance will be apparent (375). Moreover, cooling protocols vary significantly from one study to the other, in terms of starting temperature and speed of temperature change. It is expected that these factors strongly affect the responses of a channel whose activation receives both positive and negative feedback from Ca^{2+} as permeating ion (876). In addition, to explore the sensitivity of an ion channel in a physiologically meaningful way the responses to thermal stimuli should be determined starting from the physiological temperature. Fourth, the mixed pharmacology of TRPA1 with TRPM8, exemplified by the lack of specificity of menthol for the later channel, has probably resulted in the underestimation of TRPA1-mediated responses to cold (371). Fifth, some negative conclusions about TRPA1 activation by cold have been made from quite a long range, by looking at very complex *in vivo* scenarios, some times wrongly assuming full specificities of TRPA1 agonists such as cinnamaldehyde and AITC (20, 26, 124, 241, 269, 616, 677, 890). Sixth, because of its diverse regulation, TRPA1 is not expected to contribute equally to cold-sensitivity in all expression systems, pathophysiological settings and animal models. As examples, different animal strains may behave distinctly to intense cold (the jumping phenotype observed in C57BL/6J mice seems not to be present in other strains (375)), the implication of TRPA1 (and TRPM8) in cold allodynia was inferred to differ between cold injury and neuropathic pain patients (574), cold hypersensitivity induced by menthol, but not by icilin or lysophospholipids, was dependent on expression of TRPA1 (270), and the relative contribution of this channel compared to that of TRPM8 varies across different sensory neurons (244). Doubtlessly, there are multiple examples of TRPM8 as the main/sole determinant in

the responses to cold (189, 385, 401, 402, 512, 635, 644, 825, 843, 896, 948), but this does not mean that the cold-sensitivity of TRPA1 is not relevant in other scenarios.

From the mechanistic point of view, activation of mouse TRPA1 by cold (375) and *Drosophila* TRPA1 by heat (866) is associated to a negative shift of the activation curve to more negative potentials (Figure 5), an effect reminiscent of that documented for other thermosensitive TRP channels, such as TRPM8, TRPV1, TRPM4 and TRPM5 (803, 859).

The role of TRPA1 as a cold sensor has been discussed in detail (858). There is strong genetic and pharmacological evidence that activation of TRPA1 by cold translates to cellular, tissular and *in vivo* behavioral responses, especially in pathological conditions (49, 70, 104, 163, 179, 198, 219, 247, 318, 325, 355, 375, 378, 397, 427, 428, 445, 532, 533, 579, 610, 646, 680, 825, 831, 853, 919, 941, 956). However, many of these responses may not be necessarily fully mediated by direct activation of TRPA1 by cold, but indirectly through the production of a stimulatory/sensitizing factor (70, 325, 517, 532, 533, 853, 896). Evidence for functional TRPA1 expression and responsiveness to cold has been also shown in human dental pulp fibroblasts (235), human odontoblasts (236), and adenocarcinomic human alveolar basal epithelial A549 cells (784, 785). TRPA1 also contributes to cold-induced contractions in the isolated rat colon preparations (217), and is the major mediator of cold-evoked responses in mouse and rat visceral sensory neurons (244, 326, 577).

TRPA1 isoforms from fly (298, 367, 857), mosquito (298), frog (703), lizards (5, 435, 437, 703), chicken (436, 702), snakes (281, 436, 639) and the fishes medaka (Japanese rice fish) (613) and takifugu (pufferfish) (611) are activated by heat.

6.8. MECHANO-ACTIVATION

The study of the role of TRPA1 in mechanosensation was sparked by the proposal and immediate discussion of its implication in mechanosensitivity of inner-ear hair cells (174, 175, 276, 564), by the potential molecular spring properties of the N-terminal ankyrin repeats (46, 330, 759) and by the similarities between this channel and the hair cell transducer (265, 564). TRPA1 was, however, shown not to be essential for mechanosensing in hair cells (75, 445, 776, 905). To the best of our knowledge it has not been reported that recombinant mammalian TRPA1 channels can be activated by classical mechanical stimulation, i.e., membrane indentation, sheer stress or membrane stretch (see also (959)). On the other hand, there are multiple reports on the role of TRPA1 in the detection of mechanical stimuli in native cells, including sensory neurons (380), human peridontal ligament cells (836), Merkel cells (760) and odontoblasts (734). This suggests that TRPA1 is mechanosensitive only in its native environment, which is after all not surprising.

The TRPA1 inhibitor HC-030031 strongly reduces the mechanically-evoked action potential firing in rat and wild type mouse C fibers, particularly at high-intensity forces. TRPA1 inhibition does not influence the mechanical responsiveness of Aδ fiber nociceptors (380). Interestingly, TRPA1 is expressed in thin-caliber axons and intraepidermal nerve endings, as well as in large-caliber axons, lanceolate and Meissner endings and epidermal and hair follicle keratinocytes. The firing rates of action potential generated in C-fiber nociceptors in response to a wide range of forces is much lower in *Trpa1*-deficient mice than in wild type animals. Aδ mechanonociceptors of *Trpa1*-deficient mice also display reduced firing, but only to large forces. Thus, TRPA1 seems to mediate mechanotransduction via a cell-autonomous mechanism in nociceptor terminals. It is also possible that TRPA1 function in keratinocytes contributes to skin mechanosensation (447). Other studies

showed that slowly-adapting mechanically-activated currents recorded in mouse DRG neurons are inhibited by HC-030031 (856), that intermediately-adapting mechanically activated currents in neurites of mouse DRG neurons are reduced by genetic or pharmacological ablation of TRPA1, whereas rapidly- and slowly-adapting currents are unaltered (127). There is evidence for mechanosensory function of TRPA1 in *C. elegans*, where this channel is activated by application of negative, but not positive, pressure through the recording patch pipette in the whole-cell configuration (393).

The activation of TRPA1 by hypertonic solution (HTS), but not hypotonic solutions, could be also taken as argument for mechano-sensitivity of this channel (939). Interestingly, other reports are consistent with TRPA1 being activated also by hypotonic solutions in odontoblasts (234, 835) and in the heterologous expression system HEK293T cells (260).

There is evidence for activation of TRPA1 by another type of mechanical stimulation, that arising from the alteration on membrane structure by insertion of exogenous molecules. For instance, TRPA1, but not TRPV1, TRPV4 or TRPM8, is activated by trinitrophenol, a negatively charged amphiphilic compound that produces crenation of the plasma membrane upon its preferential insertion in the outer leaflet of the bilayer (315). On the other hand, chlorpromazine, which is positively charged and inserts preferentially in the inner bilayer leaflet producing membrane cupping, has no effect on TRPA1 channels with low basal activity. However, this compound induces a large increase in inward currents previously stimulated with AITC. This effect is accompanied by a complex modulation of the outward currents that seem to be consistent with an increase in the rates of channel activation and inactivation at positive potentials. In addition, GsMTx-4, a tarantula toxin that is thought to inhibit mechano-sensitive channels by inserting in the outer membrane leaflet also activates

1494 TRPA1 (315). Furthermore, primary and secondary alcohols activate human TRPA1
1495 in a carbon chain length-dependent manner, and with a strength that highly
1496 correlated with the molecule lipophilicity (416). This suggests that non-electrophilic
1497 TRPA1 agonists may act via a mechanism that involves the detection of physical
1498 alterations in the plasma membrane. This idea is further supported by the close
1499 relation between the abilities of bacterial lipopolysaccharides to activate TRPA1 and
1500 to produce mechanical perturbations in the plasma membrane (523, 764, 765).

1501 Taken together the hypothesis of intrinsic mechanosensitivity of mammalian TRPA1
1502 remains to be further investigated, but it seems clear that this channel is implicated in
1503 mechanosensation in physiological and pathological conditions (see section
1504 PATHOPHYSIOLOGY OF TRPA1).

1505 **6.9. ACTIVATION BY LIGHT**

1506 Human TRPA1 is activated by near ultraviolet (UV) light in a wavelength-dependent
1507 and membrane-delimited manner, through a mechanism that implicates the
1508 production of ROS. The sensitivity of TRPA1 to light is enhanced by the
1509 photosensitizing agents acridine orange and hypericin. These findings suggest that
1510 TRPA1 activation underlie the pain and burning sensations triggered by
1511 photodynamic therapy (316).

1512 UV light evoked a current in human epidermal melanocytes that was inhibited by
1513 ruthenium red, camphor and HC-030031 and that was about 90% reduced by
1514 TRPA1-targeted miRNA that decreased channel expression in 85%. TRPA1
1515 inhibition decreased melanin synthesis as well. The mechanism underlying TRPA1
1516 activation is not mediated by ROS production, but downstream of an opsin-mediated
1517 G protein signaling cascade involving PLC activation (82). Further studies revealed
1518 that UV-induced activation of TRPA1 result in melanocyte membrane depolarization,

which was proposed to reduce the photoactivated current inactivation, thereby prolonging the Ca^{2+} entry required for melanin production (84). Whether the cell depolarization is fully mediated by TRPA1 or also by other channels is still unresolved (142). Also obscure remains the actual contribution of the complex interplay between Ca^{2+}- and voltage-dependent gating of TRPA1 to the regulation of Ca^{2+} signaling and membrane potential in these cells. A more detailed study of the transduction signaling pathway indicates that UV light activates Gαq/11/PLCβ signaling, leading to the hydrolysis of PIP_2 and the consequent generation of diacylglycerol (DAG) and inositol 1, 4, 5-trisphosphate (IP_3). In turn, on one hand PIP_2 depletion seems to enhance TRPA1-mediated photocurrents via a relief of a tonic inhibition of channel activation, and on the other, IP_3 stimulates Ca^{2+} release from intracellular stores. Hence, melanocytes were proposed to feature a UV transduction mechanism resembling the phototransduction cascades of the eye (83).

A behavioral assay in zebrafish embryos used to screen molecules that could modify the startle reflex to light led to the identification of optovin as a reversible photoactivated agonist of TRPA1. Further experiments suggested that optovin activates human TRPA1, not via the generation of single oxygen, but partly through direct covalent modification of cysteine residues. Interestingly, treatment with optovin allowed light-mediated control of the motor activity of paralyzed extremities in spinalized zebrafishes and elicited nocifensive behaviors in mice (415). These findings have research applications in the identification of functional expression of this channel in cardiomyocytes (488) and cortex (382), and may have potential for clinical applications as a strategy for precise spatio-temporal control of endogenous TRPA1 activation (243, 415).

The clinical relevance of the implication of TRPA1 in light-induced responses was further supported by the elucidation of the molecular transduction mechanism

underlying cutaneous porphyria, a condition in which patients suffer from burning pain upon exposure to sunlight or from the effects of photodynamic therapy. Ultraviolet and blue light generates singlet oxygen that in turn acts as TRPA1 and TRPV1 agonist (56). Furthermore, human TRPA1 is activated and photosensitized by 7-DHC (7-dehydrocholesterol), a precursor of cholesterol and vitamin D_3 that is found in very high plasma levels of patients suffering from the autosomal Smith–Lemli–Opitz syndrome. TRPA1 and TRPV1 mediate responses of mouse DRG to acute application of 7-DHC in the dark and light-induced responses in the presence of 7-DHC. Illumination with 405 nm light induces release of CGRP from isolated mouse trachea, an effect that is enhanced by preapplication of 7-DHC. The latter effect is absent in preparations from double *Trpa1/Trpv1* KO animals. Finally, application of 405 nm light causes an increase in firing of mouse cutaneous C-fibers and habilitates a response to 7-DHC, which is on its own ineffective. These findings led to the proposal of a mechanism whereby TRPA1 and TRPV1 are activated by ROS, RNS and/or RCS generated from Fe^{3+}-catalyzed conversion of oxysterols that are produced by peroxydation of 7-DHC by singlet oxygen resulting from photochemical reactions involving endogenous chromophores (54). In contrast, TRPV1, but not TRPA1, appear to be implicated in the action potential firing of mouse DRG neurons triggered by protoporphyrin IX phototoxicity produced by 630 nm light (900).

The role of TRPA1 in the responses to light has received support from studies in *Drosophila*, which have shown that this channel is required for cell-autonomous light transduction in class IV dendritic arborization neurons and avoidance to light in larvae (907), for the sensitivity of neuroendocrine cells to UV light (267, 293), for rapid light-dependent feeding deterrence (220) and for blind females to avoid laying eggs under UV light (292). Furthermore, a TRPA1 homolog is required for the extraocular photophobic response of planaria to near UV light (105).

Interestingly, activation of TRPA1 and TRPV1 seems to mediate X-ray-induced mechanical and heat allodynia in mice (177).

6.10. MODULATION BY $PI(4,5)P_2$ AND POLYPHOSPHATES

Phosphatidylinositol 4,5-bisphosphate ($PI(4,5)P_2$, or PIP_2) is an acidic phospholipid of the inner leaflet of the plasma membrane comprising approximately 1% of plasma membrane phospholipids. Most if not all TRP channels are capable to interact or are indirectly modulated by PIP_2. Although PIP_2 effects on TRPA1 remain debated, it seems clear that it is involved in channel activation and desensitization (373, 389). A typical feature of TRPA1 is its rapid desensitization following activation by agonists such as AITC, CA, and high intracellular Ca^{2+} concentrations. TRPA1 desensitization is delayed when PIP_2 is supplemented via the patch pipette, whereas the PIP_2 scavenger neomycin accelerates desensitization. Pre-incubation with the PI-4 kinase inhibitor wortmannin reduces both constitutive TRPA1 channels activity and the response to AITC. These data indicate that PIP_2 modulates TRPA1, although to a lower extent than other TRP channels, such as TRPM4 (373).

Other reports showed that in inside-out patches, PIP_2 does not activate TRPA1 (389). When TRPA1 was electrophilic-activated, addition of PIP_2 produces a concentration-dependent inhibition of TRPA1. It seems that PIP_2 may act as an inhibitor of TRPA1, reducing the sensitivity of TRPA1 to its activators (389). Another study showed that capsaicin-induces cross desensitization of TRPA1 upon dialysis of PIP_2 through the patch pipette (9). On the other hand, other studies reported no effect of PIP_2 in excised patches (388) or inhibition in the presence of inorganic poly-phosphate (389). Furthermore, rapamycin induced PIP_2 depletion does not inhibit TRPA1, while it inhibits TRPM8 (876). Of note, one of the tools used to study PIP_2 signaling is the PLC inhibitor U73122, but this compound was shown to activate directly TRPA1 via

1596 covalent modification, and to induce release of CGRP from mouse skin in a TRPA1-
1597 dependent manner (373, 587).

1598 This view is further complicated by the possibility of TRPV1-TRPA1 interactions as
1599 well as by distinct experimental conditions. For instance, channel modulation by PIP_2
1600 under chronic inflammation, usually lasting many days, could be different from
1601 previously described after acute alterations within minutes. Inflammation results in a
1602 long lasting (chronic) PIP_2 depletion. Chronic PIP_2 production can be stimulated by
1603 overexpression of phosphatidylinositol-4-phosphate-5-kinase and the PIP_2 -specific
1604 phospholipid 5'-phosphatase can reduce plasma membrane levels of PIP_2. It was
1605 proposed that the responses of TRPA1 to agonists are not significantly influenced by
1606 chronic changes in PIP_2 (648). However, if TRPA1 and TRPV1 are present, chronic
1607 PIP_2 reduction leads to pronounced tachyphylaxis of both channels. Thus, the
1608 chronic effect of PIP_2 on TRPA1 activity depends on presence of the TRPV1 (648).

1609 One of the problems to study in detail effects of phosphoinositides, such as PIP_2, is
1610 the difficulty to work for long time on inside-out patches. TRPA1 seems to require an
1611 unidentified cytosolic factor whose action can be mimicked by inorganic
1612 polyphosphates. Multiple intracellular molecules are able to rescue activation of
1613 TRPA1 by covalent modification in inside-out patches. These compounds include
1614 polyphosphates (e.g. pyrophosphate, PPi)), polytriphosphates (PPPi). Application of
1615 polyphosphates to inside-out patches not only stabilizes TRPA1 activity but also
1616 increases the single channel conductance (596). Structure-function analysis of
1617 polyphosphates indicates that at least four phosphate groups are needed to render
1618 their activity (388). Finally, IP_3 and inositol-hexaphosphate partially rescue TRPA1
1619 activation. This indicates that intracellular factors are required for TRPA1 to adopt a
1620 functional channel conformation (388). It is suggested that TRPA1 can exist in
1621 different functional states: a native state (cell-attached patch) and a non-native state

(excised patch). As mentioned above, THC can activate TRPA1 even in the absence of polyphosphates, whereas electrophilic pungent chemicals and Ca^{2+} require it for activation (145).

6.11. MODULATION BY PHOSPHORYLATION

Although TRPA1 is likely to be modulated by phosphorylation, this process is not well understood and only little information is available. TRPA1 can be activated by bradykinin and *Trpa1* KO animals show impaired responses to bradykinin as well as to noxious mechanical stimuli (75). Bradykinin activates and sensitizes sensory nerves through mechanism involving G protein-coupled receptor-mediated PLC, PLA, cyclooxygenase and 12-lipoxygenase, signaling pathways. These signaling pathways subsequently interact with downstream ion channels, including TRPA1, thereby changing the excitability of sensory nerves (12, 38, 870).

Recently, possible PKA phosphorylation sites were identified in the sequence of hTRPA1 (516). The channel mutant S1101A displays increased current density, whereas the mutant S804A shows a reduction of the time to peak in response to agonist. Another mutation, S227A, results in increased PKA-mediated sensitization. Further, four mutations at positions S86, S317, S428, and S972, were shown to be possible phosphorylation sites for PKA, contributing to significant suppression of TRPA1 sensitization. These residues are highly conserved between different species, including *Drosophila*, zebrafish, mouse, rat, rhesus macaque and human (516).

The mouse TRPA1 amino acid residue S87 is a target of PKA-mediated phosphorylation. Furthermore, phosphorylation of the TRPA1 residues S119, T281, and T529 by PKC is essential for the normal sensitization of the channel. During both PKA and PKC-mediated phosphorylation of TRPA1, the scaffolding protein A-Kinase

Anchoring Protein 79/150 (AKAP) plays an essential role in anchoring these kinases to TRPA1 (123).

TRPA1 was also recently shown to have putative phosphorylation Cyclin-dependent kinase 5 (Cdk5) sites within its ankyrin repeats, which could potentially modify channel opening or conductance (782). Cdk5 phosphorylation is blocked by TFP5, a Cdk5 inhibitory peptide. These sites are highly conserved in mammals, mosquitos and fruit flies.

6.12. MODULATION BY PROTEIN – PROTEIN INTERACTIONS

The interactions of TRP channels with diverse modulatory proteins represent an increasingly important research topic (see http://trpchannel.org/). So far, the best studied examples of protein-protein interactions in this field are those between TRPV1 and the ubiquitin hydrolase CYCL (see above), the PKA anchor protein AKAP5 and secretogranin 3, a member of the chromogranin/secretogranin family of neuroendocrine secretory proteins (see also http://trpchannel.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 (8, 705). Furthermore, co-expression of these channels result in unique activation profiles that can be distinct from those of cells expressing only TRPA1 or TRPV1 (697). TRPV1 and TRPA1 function together and resiniferatoxin-mediated "neurosurgery" removes both sensor molecules. In adult mice resiniferatoxin causes desensitization to heat and sensitization to cold (653), and experiments on facial skin capsaicin injection to rats suggest that TRPV1 signaling in TG neurons sensitize TRPA1 and thereby induce cold hyperalgesia (325). Approximately 30% to 50% of TRPV1-expressing small- to medium-sized peripheral sensory neurons co-express TRPA1, and almost all TRPA1-positive neurons co-express TRPV1 (404, 777). Ca^{2+}-

triggered activation of TRPA1 is attenuated by TRPV1 in the presence of extracellular Ca^{2+}, but not in Ca^{2+}-free conditions. TRPV1 mutations at residue Y671 affect probably TRPA1 permeation properties, but the mutations in TRPV1 did not affect association of the TRPA1 and TRPV1 channels. The TRPV1 mutation Y671K alters the magnitude of currents through TRPA1, the sensitivity to extracellular [Ca^{2+}] and the voltage-dependency (649). Some TRPV1-selective cannabinoids such as WIN 55,212 are effective inhibitors of TRPA1. The synthetic cannabinoid, arachidonoly-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 (693). Conversely, knockdown of TRPA1 in sensory neurons by specific small interfering RNA abolishes the WIN effect on TRPV1 dephosphorylation, suggesting that WIN acts through TRPA1 (354). TRPA1 stimulation enhances TRPV1 phosphorylation via the putative PKA phosphorylation site S116 and this cross-sensitization contributes to higher pain sensitivity in inflamed tissues (761).

Transmembrane protein 100 (Tmem100) is a potential modulator of TRP channels, and is often co-expressed and forms complexes with TRPA1 and TRPV1 in DRG neurons. Tmem100-deficient mice display a reduction in the inflammatory mechanical hyperalgesia and TRPA1- but not TRPV1-mediated pain (888, 892). Tmem100 can selectively potentiate TRPA1 activity by weakening the TRPA1-TRPV1 association (888). In contrast, the Tmem100-3Q mutant enhances the association of TRPA1 and TRPV1 and therefore inhibits TRPA1. Notably, a cell-permeable peptide (CPP) containing the C-terminal sequence of Tmem100-3Q inhibits persistent pain, suggesting the possibility of targeting TRPA1-TRPV1 complexes for pain management (888, 892). Interestingly, TRPV1-TRPA1

interactions may explain intriguing clinical findings such as the association between a functional knockdown of TRPV1 with hypersensitivity to garlic (642).

Analysis of the effects of icilin on TRPA1 and sensitivity of mice to cold stimuli, which is inhibited by blockers of $iPLA_2$ (BEL, bromoenol lactone), suggests for a possible interaction of TRPA1 with this lipase (270).

Prokineticins PK1 and PK2, and their G-protein coupled receptors prokineticin receptor 1 (PKR1) and prokineticin receptor 2 (PKR2) play important roles in several biological processes such as gut motility, neurogenesis, angiogenesis, circadian rhythms, hematopoiesis, and nociception. *Pkr* KO animals display impaired PK1-, PK2-mediated hyperalgesia that partially depend on TRPA1 activity (588). Another link between pain and TRPA1 comes from studies on the protease activated receptor 2 (PAR2) (180, 345, 586, 931).

Annexin A2 (AnxA2) is highly expressed in the sensory neurons expressing TRPA1. Membrane TRPA1 levels and TRPA1-mediated Ca^{2+} responses are enhanced in a AnxA2-deficient mouse. *In vivo* experiments showing reduced TRPA1-dependent acute and inflammatory pain in AnxA2-deficient mice led to the conclusion that co-expression with AnxA2 in a subpopulation of sensory neurons may impair TRPA1-dependent nociceptive signaling in vertebrates (52).

Although direct interactions have not been demonstrated, pharmacological evidence suggest for functional interaction between T-type Ca^{2+} channels and TRPA1 in sensory neurons. Activation of the former results in Ca^{2+} entry that may activate and desensitize TRPA1 thereby modulating nociceptive signaling (600).

Notably, a direct binding of TRPA1 N-terminal ankyrin repeats to the C-terminal proline-rich motif of the fibroblast growth factor receptor 2 (FGFR2) activates this

receptor, resulting in lung adenocarcinoma progression and metastasis. In turn, TRPA1 is depleted by the transfer of TRPA1-targeting exosomal microRNA (miRNA-142-3p) from brain astrocytes to cancer cells (94).

6.13. TRPA1 ANTAGONISM

The huge importance of TRPA1 in pain, inflammation and many other potential indications in acquired diseases triggered an increasing demand for antagonists (410, 673, 674). Examples of these antagonists are listed in Table 5.

Table 5. Synthetic TRPA1 inhibitors

Name	Company	IC_{50}	References	Comments
HC-030031	Hydra Biosciences, Inc.: USA	≤ 1.8 - 20 μM	WO2007073505 (2007); (232, 491, 513)	Non-electrophilic. Effective in human, rat, mouse, guinea pig. Ineffective on frog and zebrafish channels (294)
Hidra 7	Hydra Biosciences, Inc.: USA	≤ 10 μM	WO2009002933 (2008)	
Chembridge-5861528	Hydra Biosciences, Inc.: USA	14 - 18 μM	(750)	
CB-625	Cubist Pharmaceuticals/Hydra Biosciences	N.D.		Phase I clinical trial completed. Discontinued due to solubility concerns (673)
Glenmark 10	Glenmark Pharmaceuticals, SA (Switzerland)	50 - 100 nM	US2009325987 (2009)	
Glenmark 15	Glenmark Pharmaceuticals, SA (Switzerland)	< 50 nM	US2009325987 (2009)	

Glenmark 37	Glenmark Pharmaceuticals, SA (Switzerland)	< 50 nM	US2009325987 (2009)	
Glenmark 17	Glenmark Pharmaceuticals, SA (Switzerland)	< 250 nM	WO2009118596 (2009)	
Glenmark 23	Glenmark Pharmaceuticals, SA (Switzerland)	0.5 - 1 μM	WO2009118596 (2009)	
Glenmark 8	Glenmark Pharmaceuticals, SA (Switzerland)	< 500 nM	WO2009144548 (2009)	
Glenmark 39	Glenmark Pharmaceuticals, SA (Switzerland)	< 500 nM	WO2009144548 (2009)	
GRC-17536	Glenmark Pharmaceuticals, SA (Switzerland)	< 10 nM	(562)	Phase IIa clinical trial (NCT01726413) Diabetic peripheral neuropathy / Respiratory disorders
2-(1,3-Dimethyl-2,4-dioxo-1,2,3,4-tetrahydro-pyrrolo[3,2-*d*]pyrimidin-5-yl)-*N*-(4-trifluoromethyl-phenyl)-acetamide (3h)	University of Ferrara	400 nM	(69)	
N,N′-bis-(2-hydroxybenzyl)-2,5- diamino-2,5-dimethylhexane	IRM LLC, A Delaware Limited Liability Company, Bermuda	N.D.	WO2007098252 (2007)	
tramadol	Grünenthal GmbH	0.1 - 10 μM	(537)	Formerly known as Tramal. First launched and marketed by Grünenthal GmbH in 1977.
AMD_09	University of Florence, Italy	10.3 - 13.2 μM	(290)	

AMD_12	University of Florence, Italy	7.3-8.2 μM	(290)	
AP-18	IRM LLC, A Delaware Limited Liability Company, Bermuda	3.1 μM	WO2007098252 (2007) (656)	
A-967079	Abbott Laboratories	67 - 290 nM	WO2009089082 (2009) (150)	
Renovis 11	Renovis, Inc. (a wholly-owned subsidiary of Evotec AG)	2.7 μM	(197)	
AZ456	AstraZeneca	30 - 305 nM	WO2012050512 (2012) (609, 844)	
AMG7160	Amgen Inc.	51 nM	(400)	
AMG2504	Amgen Inc.	35 nM	(400)	
AMG9090	Amgen Inc.	21 nM	(400)	
AMG5445	Amgen Inc.	91 nM	(400)	
AMG0902	Amgen Inc.	IC_{90} =300 nM	(461)	
CMP1	Abbott Laboratories	2 μM	(153)	
CMP2	Abbott Laboratories	1.4 μM	(153)	
CMP3	Abbott Laboratories	1.1 μM	(153)	
2B10	Amgen Inc.	90 - 260 nM	(456)	(monoclonal antibody)
SZV-1287	University of Pécs, Hungary	2.4 μM	(652)	
JNJ-41477670	Janssen Pharmaceuticals	7.2 nM	(101)	
Thiadiazole derivatives	Pfizer	0.05 – 0.93 μM	(832)	

1730

TRPA1 is inhibited by the natural compounds camphor (derived from *Cinnamomum camphoral)* (22, 39, 63, 678, 713, 914) and the related molecule borneol (731). The former compound, however, induces a rebound effect upon washout that is typical for bimodal TRPA1 modulators (22). A series of nineteen analogues (1b-5) of racemic [6]-gingerol (1a) in which three pharmacophoric regions were altered resulted in the identification of some full TRPA1 antagonists (552). Lutein is a natural tetraterpene xanthophyll, and one of 600 known naturally occurring carotenoids that incorporates into membranes. The methylated-β-cyclodextrin (RAMEB) complex of this compound reduces TRPA1 activation by electrophilic agonists (329).

Human TRPA1 currents stimulated by AITC, menthol, flufenamic acid or octanol are inhibited by 1,8-cineole (eucalyptol), a cyclic ether monoterpenoid found in eucalyptus oil. This action, together with a stimulatory effect on TRPM8 may mediate the analgesic and anti-inflammatory effects on this compound in humans. Interestingly, 1,4-cineole activates, rather than inhibits hTRPA1 (795). A subsequent study on monoterpene analogues showed that borneol, 2-methylisoborneol and fenchyl alcohol, but not norcamphor inhibit hTRPA1 activation by menthol (796). The the menthol analogue 4-isopropylcyclohexanol inhibits TRPA1, but also the Ca^{2+}-activated Cl^- channel ANO1, TRPV1, TRPV4 and TRPM8 (798).

Resolvins are anti-inflammatory and pro-resolving lipid molecules that can be endogenously produced from omega-3 polyunsaturated fatty acids such as eicosapentaenoic acid and docosahexaenoic acid. Resolvin D1 (RvD1), potently inhibits cinnamaldehyde-induced TRPA1 responses at submicromolar levels. RvD1 alleviates TRPA1-mediated acute nociception in mice triggered by intradermal hind paw injection of cinnamaldehyde or formalin. RvD1 also strongly inhibits TRPV3 and TRPV4 (64). A subsequent study revealed that RvD2 is a potent inhibitor of TRPV1 and TRPA1 in primary sensory neurons. On the other hand, RvE1 selectively inhibits

1757 TRPV1 and RvD1 is selective for TRPA1. The very interesting roles of resolvins and
1758 related lipid mediators in inflammation and pain were recently reviewed (473). Of
1759 note, hind paw inflammation experiments in male Wistar rats showed that the
1760 antinociceptive potency of RvD1 and TRPA1 blockers is weakened by either
1761 perturbation of the balance of endogenous pro- and anti-nociceptive mechanisms in
1762 inflammation or via TRPA1-opioid receptors interactions (615).

1763 The natural stilbenoids resveratrol and pinosylvin inhibit TRPA1 activation by AITC
1764 and decrease AITC-induced paw inflammation and production of the pro-
1765 inflammatory cytokine interleukin-6 (IL-6) in mice (542, 570, 930). A more recent
1766 study showed that from a series of twenty stilbenoids none modulates TRPV1 and
1767 most of them have stronger action than resveratrol, with maximal potency observed
1768 with ortho monoxygenated stilbenes 6 and 17 (572). mTRPA1 is inhibited by tannic
1769 acid (612) and by the extract of the medicinal plant *Pterodon pubescens Benth*,
1770 containing a mixture of nine sesquiterpenes and seven diterpenes (605).

1771 Several potent TRPA1 inhibitors are already available from Hydra Biosciences,
1772 Abbot, AMGEN, Glenmark, The Scripps Research Institute and Renovis. The first
1773 synthetic antagonists appeared in 2007 and were all xanthine alkaloid based, e.g. the
1774 Hydra compound HC-030031, which is the most frequently used TRPA1 antagonist.
1775 The similarity of this compound to caffeine is intriguing. Other structurally similar
1776 compounds were released by Hydra (Hydra 7, Chembridge 5861528), Glenmark
1777 (nano-molar blockers such as Glenmark 10, 15, 37). Phtalimide derivatives (high
1778 nanomolar, Glenmark 17, 34) and imidazo-ourine derivatives (Glenmark 8, 39)
1779 were released by Glenmark. All these inhibitors are non-electrophilic. Other non-
1780 electrophilic antagonists have been developed from diamino-dimethylhexanes. Other
1781 reported TRPA1 antagonists are: the analgesics tramadol and its metabolite M1
1782 (537), a novel series of α-aryl pyrrolidine sulfonamides (850) and the paracetamol

(acetaminophen) analog 6a/b (256). The antipyretic effect of paracetamol, however, is not related to its action of TRPA1 that lead to hypothermia, but to prostaglandin inhibition in the brain (529). ADM_12, a novel compound formed by a lipoic and homotaurine residues covalently linked, also antagonizes TRPA1 (585). The later reverts oxaliplatin-induced neuropathy in rats, without displaying toxicity on astrocytes or cardiotoxicity (585). The treatment of rats with the Japanese herbal medicine Gosha-jinki-gan improved oxaliplatin-induced hypersensitivity to menthol, AITC and cold, associated with suppression of mRNA TRPM8 and TRPA1 overexpression in DRG neurons (377, 540). (+)-Borneol, a bicyclic monoterpene found in the essential oil of plants used for analgesia and anesthesia in traditional Chinese medicine, induces antihyperalgesic effects in a mouse model of oxaliplatin-induced neuropathic pain, associated to the block of TRPA1 (946). A high-throughput screening of quinazolinone-based compounds that optimized antagonistic potency and increasing polarity yielded a purinone (AM-0902). This compound has pharmacokinetic properties allowing for >30-fold coverage of the rat TRPA1 IC_{50} *in vivo* and produces dose-dependent inhibition of AITC-induced flinching in rats (716).

Among electrophilic compounds, the oxime AP18 was disclosed as an antagonist of TRPA1. AP18-related oximes have both agonist and antagonist activity (197). Another oxime, A967079, is a potent nanomolar blocker of human TRPA1 that attenuates cold allodynia produced by nerve injury, but does not alter noxious cold sensation or body temperature (150). Interestingly, this compound does not inhibit frog TRPA1, which led to the identification of two amino acid residues located within the putative fifth transmembrane domain as determinants of the inhibitory action of A967079 on mammalian TRPA1 (571). More recently, 3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime (SZV-1287) was shown to inhibit both TRPA1 and TRPV1 and TRPA1-dependent CGRP release from the peripheral sensory nerve endings (652).

Other electrophilic antagonist, such as Abbott A, Renovis 11, Amgen AMG7160, 2504, 9090, 5445, and the Abbott CMP1, 2, 3, operate in the nanomolar range (678).

Pyrazolone and its derivatives dipyrone, propyphenazone and antipyrine inhibit Ca^{2+} responses and currents in TRPA1-expressing cells and mouse acute nocifensive responses induced by TRPA1 agonists. Dipyrone and propyphenazone, decrease TRPA1-mediated nociception and mechanical allodynia in models of inflammatory and neuropathic pain (91, 578). Of note, not dipyrone itself, but its metabolites 4-N-methylaminoantipyrine and 4-aminoantipyrine, activate and sensitize TRPA1 and TRPV1 in a redox-dependent manner (715).

Phα1β, a peptide from the venom of the armed spider *Phoneutria nigriventer*, and its recombinant analogue CTK 01512-2, inhibit activation of TRPA1 by AITC. This action is likely to explain how these peptides attenuate acute pain and mechanical and cold hyperalgesia elicited by AITC and the TRPA1-dependent neuropathic pain induced by the chemotherapeutic agent bortezomib (821, 869).

The quest for TRPA1 blockers is steadily increasing (see the chapters below about the role of TRPA1 in diseases).

7. PATHOPHYSIOLOGY OF TRPA1

7.1. TRPA1-RELATED CHANNELOPATHIES

So far, the only described TRPA1 channelopathy is a rare autosomal dominant Familial Episodic Pain Syndrome (FEPS) (428). FEPS appears in infancy and is characterized by episodes of incapacitating upper body pain that can occasionally radiate to the abdomen and legs. These episodes are usually triggered by fasting or

physical stresses such as cold and can last up to 90 min. FEPS patients show

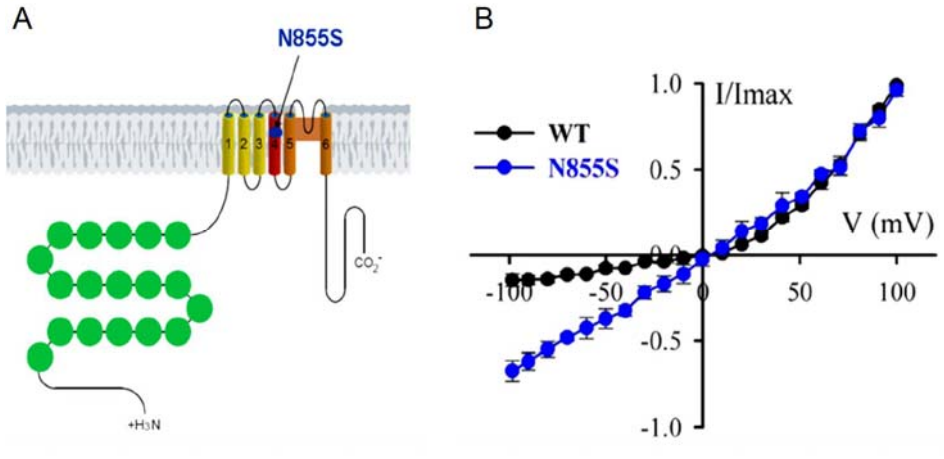

Figure 6: Structure and activity of human TRPA1 and N855S mutant underlying Familial Episodic Pain Syndrome (FEPS). (A) Schematic representation of the TRPA1 channel displaying mutation defined in the FEPS that is located in putative transmembrane segment S4. **(B)** Average current-voltage relationship of hTRPA1-WT and hTRPA1-N855S in the presence of 100 μM CA. Currents are normalized to +100 mV. Modified with permission from Kremeyer *et al*., (428).

hypersensitivity to AITC and present enhanced cutaneous flare responses and secondary hyperalgesia to punctate stimuli (428). Sequencing of candidate genes served to identify a gain-of-function missense mutation (N855S) in the S4 of TRPA1 as probable cause of the disease. This mutant channel displays a more than 5-fold larger inward current at normal resting potentials compared to the wild type channel (428) (Figure 6).

The N855 residue is involved in the Ca^{2+}-dependent activation, and the TM4-TM5 linker contributes to agonist- and voltage-dependent activation and regulates the gating of the channel in a state-dependent manner via a Ca^{2+}-sensitive mechanism (428, 954). Structural modeling and patch-clamp analysis of the N855S mutant

1843 revealed inter-subunit salt bridges between residues E854 in TM4 and K868 in TM5
1844 that stabilize the open state of the channel (954). Specific TRPA1 antagonists inhibit
1845 the abnormal *in-vitro* response of the mutant channel, suggesting that they could be
1846 used as therapy for this syndrome (428, 898). Of note, mutation of N855 to an
1847 arginine residue (N855R) was reported to enhance the response to cinnamaldehyde
1848 and the rate of inactivation, without affecting the voltage dependence of channel
1849 activation (122).

1850 Single nucleotide polymorphisms (SNPs) that may be important for somatosensory
1851 abnormalities in neuropathic pain patients have been discovered in a search for the
1852 contribution of genetic variants. A mutation, E179K, in the TRPA1 N-terminus was
1853 associated with the presence of paradoxical heat sensation (510).

1854 A study using intracellular Ca^{2+} imaging analyzed the functional properties of 4 SNP
1855 variants of human TRPA1 (Y69C, A366D, E477K and D573A) in comparison with
1856 gain-of-function channels baring the SNPs R797T and N855S (553). Only Y69C
1857 displayed enhanced sensitivity to agonists, similar to R797T, but to lower extent than
1858 N855S. The other non-conservative substitutions showed poor responses, which
1859 could be rescued by pretreatment with the Src family inhibitor PP2 or Zn^{2+}. The
1860 TRPA1 variants and several experimental mutants (TRPA1 Y97F, Y226F and
1861 YY654-655FF) expressed poorly in neuroblastoma SH-SY5Y cells compared to
1862 HEK293 cells.

1863 Another study aimed at determining the influence of human SNPs on the responses
1864 of TRPA1 to known chemical agonists and components of diesel exhaust particles
1865 and insoluble coal fly ash (CFA) particles. The variants R3C and R58T, present in the
1866 N terminus displayed gain-of-function, with enhanced responses to these stimuli
1867 (196). Several variants such as E179K and K186N located in the ankyrin repeat

1868 domain-4 and at the predicted N-linked glycosylation site residues N747A and N753A
1869 showed reduced response to CFA (196). These findings suggest for roles for ankyrin
1870 domain-4 and cell surface N-linked glycans in the mechanism underlying the
1871 activation of TRPA1 by insoluble particles. Importantly, the polymorphisms R3C and
1872 R58T correlated with reduced asthma control in children, suggesting for a link
1873 between TRPA1 and the severity of asthma in the context of high air pollution (196).

1874 The TRPA1 agonist menthol is often used in commercial nicotine-containing products
1875 to attenuate the sensation of burn associated with nicotine (384, 801). Preference for
1876 smoking menthol cigarettes is variable between individuals and populations,
1877 suggesting that differences in the *Trpa1* gene could contribute to the preference of
1878 mentholated products. A common *Trpa1* haplotype defined by 1 missense and 10
1879 intronic SNPs is associated with preference for mentholated cigarettes in heavy
1880 smokers (838).

1881 Future studies should be directed at understanding the structural bases of the
1882 perturbed functional properties of TRPA1 variants associated to human disease.
1883 Likewise, a more comprehensive characterization of the effects of channel
1884 modulators should be perfomed for these variants. Altogether, this may serve to
1885 design therapeutic strategies aimed at correcting TRPA1 channelopaties.

1886 **7.2. TRPA1 IN PAIN AND INFLAMMATION**

1887 The sensation of pain results from somatosensory stimuli generating a cascade of
1888 adaptive responses in the body. Feeling pain is essential for our survival as it
1889 provides a warning signal. This sensation involves a complex interaction between
1890 specialized nerves, the spinal cord and the brain. The detection of painful
1891 mechanical, thermal or chemical stimuli is attributed to the activation of nociceptors
1892 present in the primary afferent nerve fibers of the somatosensory system (358, 854).

Experiments in the skin indicate that chemical activation of TRPA1 produces pain, heat sensation and mechanical hyperalgesia, cold hyperalgesia and a neurogenic axon reflex erythema (258, 541, 575). A sustained activation of TRPA1 by endogenous agonists (408) implicates this channel in persistent and chronic pain in a wide variety of conditions, such as neuropathy, inflammation, osteoartritis, migraine, diabetes, fibromyalgia, bronchitis and emphysema (581, 855, 959) and therefore may be an excellent target for novel analgesic and anti-inflammatory molecules (39, 203, 266).

7.2.1. TRPA1 IN NEUROPATHIC PAIN

Peripheral sensory neuropathy is a neurological deficit that may result in decreased sensation of the peripheral nervous system (338). Patients with this condition present symptoms of pain, decreased or loss of touch, vibration, and thermal sensation (337). A loss of protective sensation puts patients at risk of undetected injury. The management of neuropathy related pain symptoms is challenging, as it often involves strong opioid containing drugs that could lead to dependence and addiction.

TRPA1 mediates pain evoked by mechanical stimuli in peripheral neuropathy and block of this channel reduces mechanical hypersensitivity induced by peripheral diabetic neuropathy (409, 879, 880) or spinal nerve injury (232, 881). In the diabetic and spared nerve injury (SNI) models of neuropathy a selective TRPA1 inhibitor, Chembridge-5861528 (CHEM) fails to induce conditioned place-preference (CPP) (882). In another study, systemically administered morphine and pregabalin reduced mechanical hyperalgesia and the spontaneous discharge rate of the presumed pain-relay neurons of diabetic animals, without inducing CPP (694). This indicates that ongoing pain, as revealed by CPP, is less sensitive to treatment by the TRPA1 channel antagonist than mechanical hypersensitivity in peripheral neuropathy (882).

1918 It was latter shown that prolonged peri-injury block of spinal TRPA1 with CHEM
1919 attenuates maintenance, but not development of mechanical allodynia following
1920 nerve injury (883).

1921 More evidence of TRPA1 function in the development of neuropathic pain was
1922 obtained from a rat model of a spinal nerve ligation in which administration of diluted
1923 bee venom to the spinal nerves reduced the expression of a few TRP channels,
1924 including TRPV1, TRPA1, TRPM8, and c-Fos in the ipsilateral dorsal root ganglion.
1925 This treatment also decreased the development of the mechanical, thermal and cold
1926 allodynia (406). Interestingly, the expression levels of TRPA1 mRNA in intact nerves
1927 adjacent to the injured nerves are up-regulated, indicating their possible contribution
1928 to development of neuropathic pain (682). Artemin, a neurotrophic factor derived
1929 from glial cells, inhibits the TRPA1 activity and pain behaviors upon short term
1930 application (929), but in long term increases the expression of TRPV1 and TRPA1 in
1931 cultured DRG neurons (341). Peripheral nerve injuries induce pain and
1932 hypersensitivity that can be attenuated by blocking TRPA1 channels (232, 610).
1933 Recently, it was described that microinjections of antioxidants or selective TRPA1
1934 antagonists into the amygdala decrease pain and mechanical hypersensitivity in
1935 nerve-injured animals (698). Along the same line, the pain-like behaviours elicited by
1936 a constriction of the infraorbital nerve mouse model are fully mediated by TRPA1, via
1937 oxidative stress by-products released from monocytes and macrophages recruited at
1938 the injury site (826). Notably, a subsequest study on mice with partial sciatic nerve
1939 ligation reported that TRPA1 channel expressed in Schwann cells, and not those in
1940 nociceptos, are the ones activated by the NADPH oxidase 2 (NOX2)-dependent
1941 oxidative burst produced by macrophages recruited to the perineural space. In turn,
1942 activation of TRPA1 in Schwann cells stimulates the NOX1 pathway resulting in the
1943 release of H_2O_2 that ultimately activates TRPA1 in nociceptors (187). Notably, TRPA1

1944 activation in these cells by the ethanol metabolite acetaldehyde initiates a NOX1-
1945 dependent production of H_2O_2 and 4-hydroxynonenal, which in turn may induce
1946 allodynia via activation of this channel in nociceptors. These findings suggest that
1947 TRPA1 in Schwann cells mediate some symptoms of ethanol-related pain (185).

1948 The sensitivity of TRPA1 to reactive oxygen species was proposed to mediate also
1949 the key role of this channel in a mouse model of neuropathic pain induced by sciatic
1950 nerve chronic constriction (662). This model features a supporting involvent of the
1951 sympathetic nervous system via α-adrenoreceptors that leads to hypersensitivity to
1952 mechanical stimulation, cold and local injection of AITC or norepinephrine. Similarly,
1953 H_2O_2-induced activation of TRPA1 was proposed to mediate the inflammatory
1954 response in an acute gout attack rodent model (827).

1955 The vasoactive peptide endothelin-1 has been suggested as a target for pain
1956 treatment, as it is implicated in several pain conditions related to inflammation,
1957 cancer, diabetic neuropathy, and sickle cell disease (749). TRPA1 inhibition reduces
1958 the pain-like behaviours induced by intraplantar injections of endothelin-1 in mice,
1959 possibly via a PLC-dependent and PKC-independent pathway. Furthermore,
1960 endothelin-1 enhances the pain induced by cinnamaldehyde (469), which could result
1961 from a sensitization of TRPA1 via the endothelin A receptor
1962 and protein kinase A pathway (942), and/or from the its stimulatory effect on TTX-
1963 resistant voltage-gated Na^+ channels in DRG neurons (950). TRPA1 seems to be
1964 also implicated in the spontaneous pain behavior elicited by acid-induced oral ulcer in
1965 the rat, a model in which endothelin-1 signaling plays a key role (601).

1966 Anticancer treatments with bortezomib, oxaliplatin, cisplatin and paclitaxel, elicit
1967 severe cold and mechanical allodynia via oxidative stress-dependent TRPA1
1968 activation (526, 579, 828). Blocking TRPA1 attenuates cold and mechanical allodynia

induced by anti-cancer drugs, such as paclitaxel or oxaliplatin (155, 579, 585). Oxaliplatin and cisplatin increase TRPA1 mRNA expression in rodent TG and DRG neurons (163, 526, 790, 817, 919). Recent studies postulate that the acute hypersensitivity to cold induced by oxaliplatin is mediated by human TRPA1 sensitization to ROS via mechanisms that, depending on the dose, are mediated by inhibition of propyl hydroxylases (533, 567) or by oxidation of cysteine residues (532). Pharmacological evidence indicates that TRPA1 is implicated in abnormal local cold-induced vascular responses observed during oxaliplatin-induced peripheral neuropathy (636). It must be noted, however, that these compounds act on several other ion channels controlling neuronal excitation and cold sensing: TREK1, TRAAK and HCN (204), TRPM8 (402, 538) and $Na_V1.6$ (205). Treatment with oxaliplatin enhances the responses of adult rat DRG neurons to icilin, but not to the TRPM8 agonist WS-12, suggesting for sensitization of TRPA1 (28). Recently, oxaliplatin was shown to induce an intracellular acidification in DRG neurons (683). As described above, changes in the cytosolic pH are linked to a sensitization of TRPA1 channels, suggesting for another mechanism of oxaliplatin induced neurotoxicity.

Paclitaxel-evoked mechanical allodynia is partially reduced by the TRPA1 antagonist (HC-030031) and the TRPV4 antagonist (HC-067047) and fully attenuated by the combination of both inhibitors (507). Paclitaxel treatment induces release of CGRP and acts via oxygen radical formation, that in turn target TRPA1 and TRPV4. Whereas TRPA1 and TRPV4 are needed for the delayed development of mechanical allodynia, the cold allodynia is exclusively dependent on TRPA1 activity (507). Treatment of cultured DRG neurons with paclitaxel at low concentration of paclitaxel (10 nM) increases CGRP release, but at a 30-fold higher concentration (300 nM) it induces the opposite effect (665).

TRPA1 is also a key mediator of the proinflammatory/proalgesic effects of exemestane, letrozole and anastrozole, three aromatase inhibitors used in breast cancer therapy (262), and of the aromatase substrate androstenedione (188). TRPA1 is activated also by two other compounds with chemotherapeutic potential: the lipoxygenase inhibitor and antioxidant nordihydroguaiaretic acid (NDGA) and its derivative terameprocol. When injected in mice none of these compounds induce spontaneous pain behaviors, but enhance the responses to evaporative cooling induced by topical application of acetone (679). Vinca alkaloids, anti-mitotic and anti-microtubule compounds derived from the periwinkle plant *Catharanthus roseus*, also induce TRPA1 activation, thereby inducing an immediate pain syndrome in fruitflies and mice (114).

2005 The evidence for the roles of TRP channels in chemotherapy-induced peripheral pain

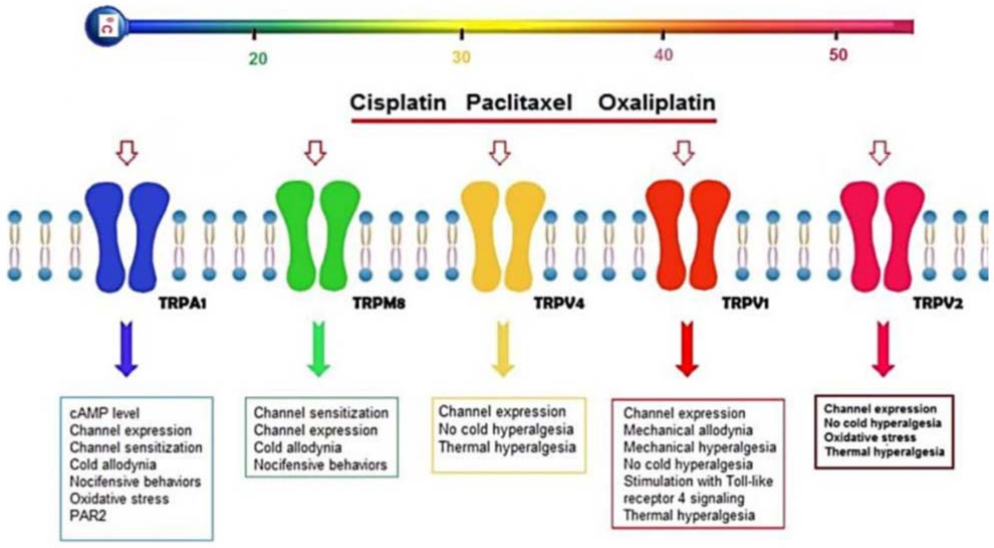

Figure 7: Summary of chemotherapeutic agents effects such as cisplatin, oxaliplatin, and paclitaxel on TRP channels (TRPA1, TRPM8, TRPV1, TRPV2, and TRPV4) in the DRG neurons. Activation of the TRP channels by chemotherapeutic can induces changes in the channels expression levels, nociceptive behaviors, oxidative stress, mechanical, heat and cold hypersensitivity. Moreover, channel levels are influenced by activation of some secondary molecular mechanisms such as glutathione (GSH) or proteinase-activated receptor 2 (PAR2). The pro-inflammatory agents trypsin and mast cell tryptase cleave PAR2, inducing its activation. PAR2 is expressed in sensory nerves and functionally coupled with TRPA1 channel. PAR2 activation was shown to increase the TRPA1 currents in native DRG neurons. Modified with permission from

2006 has been recently reviewed (586) (Figure 7).

2007 **7.2.2. TRPA1 AND INFLAMMATION**

2008 TRPA1 is a main player in the fast onset and maintenance of inflammation. Many

2009 endogenous TRPA1 agonists are produced during inflammation, e.g., as the result of

2010 lipid oxidation, peroxide formation, oxidative stress (e.g. oxygen ions, free radicals
2011 and peroxides), unsaturated aldehydes (e.g. HNE), electrophilic prostaglandins and

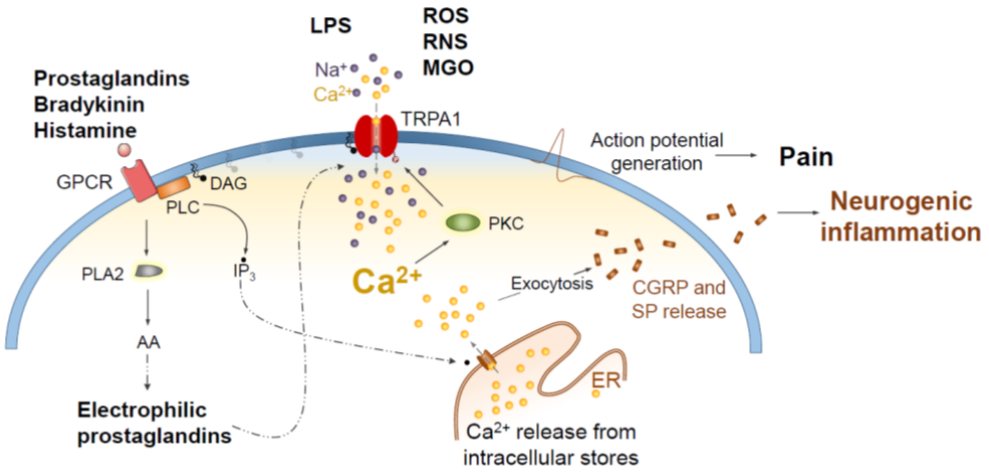

Figure 8: Activation and sensitization of TRPA1 by inflammatory mediators. Activation of the G protein-coupled receptor (GPCR) stimulates phospholipase C (PLC), with the ensuing formation of 1,4,5-trisphosphate (IP_3) and diaglycerol (DAG). IP_3 further activates the IP_3R, inducing the release of Ca^{2+} from the ER. The hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP_2) releases the inhibitory effect over TRPA1. DAG and Ca^{2+}-mediated protein kinase C (PKC) activation sensitizes TRPA1. In parallel, GPCRs activate phospholipase A2 (PLA2) leading to the downstream formation of arachidonic acid (AA) metabolites, which are endogenous activators of TRPA1. The rise ion intracellular Ca^{2+} triggers the release of inflammatory mediators (i.e., CGRP). In the inflammatory context, TRPA1 is directly activated by reactive oxygen (ROS) and nitrogen (RNS) species, methylglyoxal (MGO) or lipopolysaccharides derived from invading gram-negative bacteria. TRPA1 activation leads to the generation of action potentials in sensory neurons, triggering acute pain sensation.

2012 RNS (279, 829) (Figure 8).

2013 TRPA1 is linked to occurrence of the inflammatory symptoms of rosacea (48). Along
2014 the same line, a recent study revealed the implication of TRPA1 in the
2015 immunomodulatory effects of cinnamaldehyde in an in vivo mouse model of LPS-
2016 induced systemic inflammatory response syndrome (519).

2017 Further, TRPA1 activation in epidermal keratinocytes enhances the expression of
2018 pro-inflammatory cytokines known to be key contributors to skin inflammation (47).
2019 By triggering the release of CGRP, TRPA1 activation causes vasodilation, a key
2020 symptom in inflamed tissue (432). Inflammation is also mediated by TRPA1 via
2021 increase in the levels of TNFα, a key player in joint inflammation. Blockade of TRPA1
2022 may be therefore beneficial in reducing chronic arthritic pain (248).

2023 In the context of tissue wound healing in a mouse cornea alkali burn injury model it
2024 was found that lack of TRPA1 in cultured ocular fibroblasts attenuates the expression
2025 of transforming growth factor β1, interleukin-6, and α-smooth muscle actin. TRPA1
2026 ablation results in reduced inflammation and fibrosis/scarring in the corneal stroma
2027 during wound healing (626). At the level of the skin, it was recently shown that HC-
2028 030031 has antinociceptive and anti-inflammatory effects after thermal injury (183,
2029 287).

2030 TRPA1 is expressed on astrocytes in the central nervous system (457, 735, 736,
2031 823, 849), where it is involved in the spontaneous peptide hormone exocytosis (800)
2032 and its ablation decreases mature oligodendrocyte apoptosis and thereby
2033 demyelination and behavioural alterations and morphological changes induced by
2034 cuprizone (115, 700). TRPA1 activation may regulate mitogen-activated protein
2035 kinase pathways, the transcription factor c-Jun and a proapoptotic Bcl-2 family
2036 member (Bak). Inhibition of TRPA1 may therefore be a strategy to treat multiple
2037 sclerosis and to limit demyelination and consequent damage of the central nervous

system (700). Furthermore, TRPA1 inhibition decreases myelin damage during ischaemia, suggesting this channel as a therapeutic target in white matter ischaemia (299). TRPA1 seem to be implicated in neuronal development and oligodendrocyte maturation, theby regulating emotion, cognition, learning, memory, and social behavior (455). Interestingly, TRPA1 mediates the stimulatory effect of oligomeric forms of the amyloid-beta peptide on intracellular Ca^{2+} in mouse astrocytes and the resulting synaptic dysfunction, which may suggest a role of this channel in Alzheimer's disease (121).

Psoriasiform dermatitis induced by imiquimod, an immune regulator with antitumoral activity, is increased by ablation of TRPA1 in mice. Although this compound was reported to induced Ca^{2+} influx in TRPA1-expressing cell lines and the channel was found to be expressed in CD4+ T helper cells (379), the mechanism underlying the protective role of TRPA1 in this model of psoriasiform dermatitis remains unclear.

7.2.3. TRPA1 IN OSTEOARTHRITIS

Osteoarthritis is a common disorder characterized by the progressive loss of articular cartilage and remodeling of the underlying bone. Typically, small joints of the hands and feet, the hips, and the knees are the most affected by the disease. Osteoarthritis induces damage of articular cartilage, formation of new bone in the subchondral region, and formation of new bone and cartilage at the joint margins (154, 504). The chronic low-grade joint inflammation in osteoarthritis highly contributes to disease development and progression (500).

Cartilage degradation is caused by changes in the production of catabolic, anabolic, and inflammatory mediators within the joint, induced by increased expression of matrix-degrading metalloproteinases (MMPs) and proinflammatory mediators such as interleukin IL-6 and PGE2 (370). Monosodium iodoacetate (MIA)-induced arthritis is

commonly used as a model of osteoarthritis. MIA-induced inflammation and degenerative cartilage changes and joint pain are decreased in TRPA1 deficient animals (511, 543). TRPA1 is functionally expressed in primary human osteoarthritis chondrocytes and in the human T/C28a2 chondrocyte cell line. This channel also mediates the production of osteoarthritis-related factors such as matrix metalloproteinase-1 (MMP-1), MMP-3, MMP-13, IL-6, and PGE2 as evidenced by pharmacological inhibition and genetic depletion of TRPA1 (608). Pharmacological block of TRPA1 reduces mechanical hypersensitivity in nociceptive neurons of the spinal dorsal horn (511), whereas pain sensation is sustained (629). This suggests for a role of TRPA1 in mechanical hypersensitivity rather than in ongoing pain in osteoarthritic animals.

7.2.4. TRPA1 IN MIGRAINE

Migraine is a neurovascular disease characterized by episodic attacks of predominantly unilateral headache, often associated with nausea, vomiting and hypersensitivity to light, sound and odors. Trigeminovascular system activation, followed by dural neurogenic inflammation and sensitization are linked to development of migraine attacks. Several mediators, including CGRP and substance P are important factors in migraine-associated symptoms (201).

TRPA1 and TRPV1 play crucial role in the migraine pathophysiology (88, 201, 581). The implication of TRPA1 is strongly supported by its sensitivity to exogenous and endogenous agents that are known to trigger migraine and by the reduction of the activity of this channel by antimigraine agents (via inhibition or desensitization) (89, 90). In the migraine context, TRPA1 stimulation may occur at the level of dural afferent fibers (335). This may be via direct chemical stimulation, as reported for umbellulone (231), or via local oxidative, nitrative, carbonylic and electrophilic stress

(88, 421, 728). In some cases, TRPA1 was shown to be associated with the release of vasodilating CGRP.

It was recently proposed that migraine is a homeostatic protective response to brain oxidative stress that is mainly detected by TRPA1 (120). It must be noted that the contribution of the modulation of TRPA1 by H_2S and NO to headache generation is not straightforward. Spinal TG neurons are activated by HNO derived from these chemicals, whereas activation of meningeal afferents by HNO may produce reduced spinal TG activity, due to a rise in the electrical threshold caused by TRPA1 activation in afferent fibers (810). Stimulation of TRPA1 leading to migraine may also occur peripherally, through the effect of environmental irritants, such as inhaled acrolein, which acutely increase meningeal blood flow via the trigeminovascular system (433, 434). Of note, inhaled acrolein acts on TRPA1 in the periphery producing lipidic imbalance in trigeminal tissue and enhanced levels of modulators of TRPV1, TRPV3 and TRPV4 (462). The TRPA1-mediated signaling in meningeal afferents was proposed to be increased by obesity (502). Interestingly, the antimigraine effect of parthenolide, a major component of feverfew, is related to partial agonism and desensitization of TRPA1 (506). Similar findings were reported for the major component of butterbur (*Petasites hybridus*), isopetasin (89). Recent studies on the therapeutic action of extracranially applied botulinium neurotoxins on migraine indicate that their action may be mediated by defunctionalization of TRPA1- and TRPV1-expressing meningeal nociceptors (938). Furthermore, pharmacological evidence obtained with the use of umbellulone and AITC show that TRPA1 activity and CGRP are crucial for the regulation of cortical spreading depression, an underlying cause of migraine aura (356). Migraines are also increased in patients who have experienced infantile colic (689). As TRPA1 is the last from sensory TRPs

to be expressed during development (319), it could be speculated that human TRPA1 expression coincides temporally with the emergence of colic symptoms.

7.2.5. TRPA1 IN ORO/FACIAL/DENTAL PAIN

Odontalgia (toothache) is one of the most common types of pain induced by inflammation of the dental pulp (pulpitis).

One of the main features of dental pulp inflammation is hypersensitivity to cold. The oro-dental system is innervated by trigeminal branches expressing multiple receptors that are involved in the detection and transduction of noxious and non-noxious thermal stimuli, including TRPM8 and TRPA1 channels. TRPA1 plays a crucial function in the oro-dental system and increase in the channel expression has been linked with hyperalgesia and allodynia following tooth injury (295). *In vivo* and *in vitro* administration of nerve growth factor (NGF) increases TRPA1 mRNA and TRPA1 protein expression in a concentration- and time-dependent fashion in TG neurons (212). These findings indicate that elevated TRPA1 expression induces cold hyperalgesia and bradykinin-induced acute thermal hyperalgesia in the orofacial region.

Enhanced expression of TRPA1 and TRPV1 channels in TG neurons was also described in a model of orofacial pain based on skin incisions on the oral mucosa or the whisker pad skin. This again leads to mechanical allodynia and hyperalgesia to cold (840).

Pain associated with stimulation of a sensitive tooth involves the mechanisms of mechanotransduction. The majority of pulpal afferents express acid-sensing ion channel 3 (ASIC3) and TRPA1, suggesting these channels as targets for the treatment of dentin sensitivity (310).

Odontoblasts are cells of the outermost cellular layer of the dental pulp. These cells have as main function the dentinogenesis, but are also implicated in tooth pain (494). Odontoblasts have cytoplasmic extensions throughout the dentin and locate within the dentinal tubules. They lie in close proximity to sensory unmyelinated nerve fibers that project from the dental pulp to the inner half of the dentin and may sense and transduce external stimuli (136). Odontoblasts express several classes of ion channels, such as L-type Ca^{2+} channels, mechanosensitive K^+ channels and voltage-gated Na^+ channels (18, 495). Human odontoblasts also highly express TRPA1, TRPV1 and TRPM8, which play crucial roles in the detection of chemical and thermal stimuli. TRPV1 and TRPA1 co-localize with peptidergic sensory neurons expressing substance P and CGRP, indicating the role of TRPA1 and TRPV1 in neurogenic inflammation (235, 236).

Pain and allodynia related to mechanical trauma in the oral mucosa is linked to prostanoid- and PAR2-dependent activation of TRP channels. In oral mucositis, spontaneous pain occurs due to TRPV1 and TRPA1 activation and mechanical allodynia through TRPV4 activity, independently of bacterial infection (345). Moreover, inhibition of TRPA1 alleviated mechanical allodynia associated with the action of bacterial toxins in oral ulcerative mucositis induced by systemic administration of the chemotherapeutic drug 5-fluorouracil (918).

Some tooth whitening (bleaching) treatments employ hydrogen peroxide at high concentrations (up to 35%) to oxidize chromogens in the dentin. Other whitening systems use carbamide (urea) peroxide. Patients undergoing bleaching procedures often complain of painful sensations that are referred to as “bleaching sensitivity” (BS) (311, 463). Bleaching products quickly diffuse into the dental tissues reaching and activating TRPA1 channels in the intradental nerves, causing BS pain (503). Post-bleaching sensitivity is also related to the morphological changes that

presumably enhance the tissue permeability, resulting in transient sensitivity after the whitening procedure.

In the search for novel pharmaceutical TRPA1 analgesics compound, AZ465, was identified as a reducer of CGRP release from human dental pulp (609). Administration of the TRPA1 inhibitor HC-030031 attenuated mechanical allodynia and cold hyperalgesia in a model of orofacial pain based on skin incisions on the oral mucosa or the whisker pad skin (840). HC-030031 also decreases spontaneous guarding pain behavior elicited by skin and deep tissue incision in mice (781), a phenomenon that seems to be related to the strong response of muscle tissue to H_2O_2 (780).

7.2.6. TRPA1 IN BACTERIAL INFECTIONS

Bacterial infections, as well as injections the complete form of the Freund´s adjuvant mycobacteria mixture (CFA), are well known to induce acute pain, mechanical allodynia, tissue swelling and chronic inflammation (248). Numerous reports demonstrate that the CFA-induced inflammatory pain is attenuated by administration of TRPA1 inhibitors and in *Trpa1* KO mice (328, 511, 656).

Recent studies have shown that LPS activates sensory TRP channels (117), including several TRPA1 isoforms: mouse, human (523) and *Drosophila melanogaster* (754). LPS activates TRPA1 in mouse sensory neurons in a TLR4-independent manner, and genetic ablation of this channel reduces mouse pain and inflammatory responses triggered by LPS (523). In fruit flies, TRPA1 expressed in bitter-sensing neurons mediates gustatory avoidance to LPS (754). Of note, LPS also activates TRPV4 channels in airway epithelial cells (21), and with lower potency

TRPV1, TRPM3 and TRPM8 (118). The mechanism of activation of TRPA1 by LPS seems to be related to the ability of this molecule to induce mechanical perturbations in the plasma membrane (765). Moreover, it was demonstrated that LPSs with diverse conformations of lipid A, such as LPS from *E. coli* or *S. minnesota*, have different potencies in inducing changes in the membrane order that relate to their ability to activate TRPA1 (765).

The pathophysiological mechanisms underlying low back pain and enhanced pain in diabetes was linked with low-grade bacterially induced inflammation (13). Furthermore, patients with chronic low back pain triggered by lumbar disc herniation display elevated plasma levels of methylglyoxal (480), an agonist of TRPA1 expressed in human mechano-insensitive C fibers (223), which suggests this channel as a potential treatment target in these patients.

Interestingly, there seems to be a link between TRPA1 and viral infections as well, as the expression of this channel is upregulated dIMR-32 neuronal cells by soluble factors released during HRV replication in the Wi-38 lung cells (1).

7.3. TRPA1 AND ITCH

Itch (or pruritus) is an unpleasant sensation provoking scratching behavior (909), leading to decreased life quality (7, 78, 478). Itch is related to eczema, psoriasis, urticaria, renal failure, cholestasis, lymphoma, and chronic liver diseases (909). The scratching reflex is associated with sensitization of nerves sensing exogenous pruritogens and endogenous chemicals produced by epithelial and immune cells.

Itch is related to TRPA1 activation, but the underlying sensation is different from pain (395, 514). Mas-related G protein-coupled receptors (Mrgpr) constitute a class of histamine-independent receptors activated by mast cell mediators and chloroquine

(285, 478). MrgprA3 and MrgprC11 act upstream of TRPA1 and their expression overlaps with that of TRPA1 and TRPV1 (342, 894). Of note MrgprD is required for cold allodynia in neuropathic pain induced by chronic constriction injury via a PKA-TRPA1 pathway, but the itching behavior is unaltered in this experimental model (865). TRPA1-deficient mice display reduces the scratching behavior in response to chloroquine and BAM8–22, implicating TRPA1 in histamine-independent pruritus (894). Although BAM activates TRPA1 and TRPV1, only TRPA1 is required for BAM-evoked itch-related behaviors. BAM-induced TRPA1 activation is linked to PLC activity, but inhibition of PLC does not alter chloroquine-evoked activation of TRPA1 (894). Mice lacking PLCβ respond to chloroquine with normal itch behavior related to TRPA1 but not to TRPV1 activation (342). It was recently reported that chloroquine-evoked scratching responses in TRPA1-deficient mice do not differ from those of WT animals. Instead, evidence was provided for a role of TMEM16a Ca^{2+}-activated Cl^- channels in chloroquine-induced increases in sensory neuron excitability and scratching (692).

Serotonin (5-hydroxytryptamine, 5-HT) is released by mast cells and is associated to atopic dermatitis, cholestasis and psoriasis (752). Serotonin receptor (HTR7) stimulation leads to TRPA1 activation, triggering itch behaviors (559). Mice lacking HTR7 or TRPA1 display reduced scratching and skin lesion severity, highlighting their role in acute and chronic itch (559). HTR7-linked activation of TRPA1 requires functional adenylate cyclase (AC), and TRPA1 is sensitized by AC and cAMP in sensory neurons (559, 717). Remarkably, TRPA1 does not couple to all HTRs. HTR2 activation by α-methyl 5-HT triggers itch and pain behaviors that are independent of TRPA1 (478, 717, 894).

As mentioned above, TRPA1 inhibition reduces the pain induced by endothelin-1. However, TRPA1 inhibition exacerbates the scratching behavior of mice triggered by

intradermal injection of this peptide, but the mechanism underlying this effect remains unknown (470).

In the context of cholestatic hepatitis and other obstructive liver disease (464), it was found that activation of G-protein–coupled bile acids receptor 1 (TGR5) expressed in primary sensory neurons sensitizes TRPA1 via a Gβγ- and PKC-dependent mechanism (472). TRPA1 inhibition prevents bile acid-stimulated release of the pruritogenic neuropeptide gastrin-releasing peptide (GRP), and decreases bile acid-induced scratching. Furthermore, spontaneous scratching induced by endogenous bile acids in mice overexpressing TGR5 is reduced by TRPA1 inhibition (472).

Atopic dermatitis (AD) is a chronic itch and skin inflammatory disorder characterized by intolerable and incurable itch (307, 483, 762, 952). The protease-activated receptor 2 (PAR2) plays an important role in the production of the cytokine thymic stromal lymphopoietin (TSLP), which triggers AD (545, 952). TSLP is expressed in

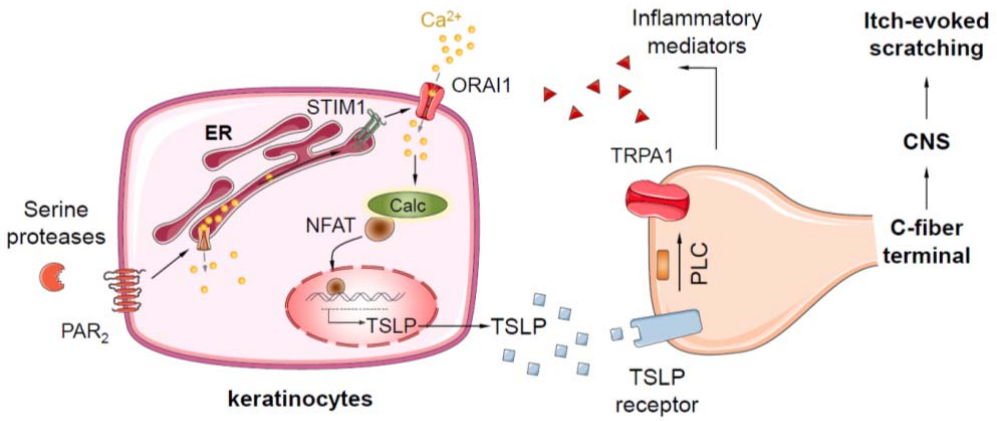

Figure 9: Schematic diagram depicting the thymic stromal lymphopoietin (TSLP)-mediated sensitization of TRPA1 during chronic itch. ORAI1/NFAT-dependent release of TSLP activates the TSLP receptor in innervating cutaneous sensory neurons. Keratinocyte-secreted TSLP depolarizes a subset of C-fibers thereby producing itch in a TRPA1-dependent manner. Activation of TRPA1 in sensory neurons leads to neurogenic inflammation through the release of neuropeptides in the skin.

human cutaneous epithelial cells in AD and bronchial epithelial cells in asthma (352). TRPA1 is required for TSLP-evoked sensory neuron activation, triggering robust itch-evoked scratching, involving PLC signaling (895) (Figure 9).

Interleukin-13 (IL-13) is one of the critical mediators of AD (620). This Th2 cytokine enhances the growth of dermal neuropeptide-secreting afferent nerves and enhances the expression of TRPA1 in dermal sensory nerve fibers, DRG neurons and mast cells. TRPA1 ablation decreases scratching behaviors (620). Another cytokine, IL-31, is implicated in inflammatory and lymphoma-associated itch (113, 182) and induces itch by activating IL-31RA in the skin sensory nerves expressing TRPA1 and TRPV1. IL-31-induced itch is highly reduced in TRPV1- and TRPA1-deficient mice but not in c-kit or proteinase-activated receptor 2 mice (148). Thus, IL-31RA signaling links neuro-immune crosstalk between T cells and sensory nerves.

The lymphoma-associated itch is suppressed by a miRNA-711 inhibitor and a blocking peptide that disrupts miRNA-711/TRPA1 interaction. Extracellular miRNA directly activates TRPA1 channels inducing TRPA1-depedent itch, confirming miRNAs role in pruritogensis (300, 729).

TRPA1 is a major integrator of histamine-independent inflammatory and pruritogenic signals in oxazolone-induced contact dermatitis in mice. TRPA1 deletion reduces the levels of the proinflammatory cytokines IL-4, IL-6, and CXCL-2. TRPA1-deficient animals display reduced skin edema, keratinocyte hyperplasia, nerve growth, leukocyte infiltration, and antihistamine-resistant scratching behavior once exposed to the haptens oxazolone and urushiol (478). Topical application of xylene and toluene in mouse induces edematogenic and nociceptive responses that are prevented by HC-030031 and by the genetic deletion of TRPA1. TRPA1 activation

may be therefore implicated in some of the symptoms of irritant-mediated contact dermatitis (602).

Phthalates are found in many consumer and industrial products (366), but increase the risk of developing different allergies (407, 449) and asthma (95). It was proposed that exposure to di-isononyl phthalate enhances expression and/or activation of TRPA1 via NF-κB signaling. This induces higher levels of IL-6 and Th2 cytokines, stimulating the development of ADC in mice (366). TRPA1 activation correlates with the enhancing effect of phthalates used in industry and as components of mosquito repellents in a fluorescein isothiocyanate model of contact hypersensitivity (442-444, 732, 733). In a follow-up study it was found that esters with glycerol and a short chain fatty acid, dibutyrin and tributyrin, activate TRPA1 and enhanced skin sensitization to FITC (724).

TRPA1 mediates the itch-related mouse behaviors elicited by cheek injection of lysophosphatidic acid. This compound activates both TRPA1 (and TRPV1) when applied intracellularly requiring the amino acid pairs K672-K673 and K977-R978 (396).

Taken together, the evidence gathered so far in animal models strongly indicates for the implication of TRPA1 in the trigger and/or maintenance of pain and inflammatory conditions. However, clinical data remains scarce. Due to its accesibility and its relative isolation from the internal tissues of the body, the skin is probably the most tractable organ for the study of the pathophysiological roles of TRPA1 in humans. The available evidence for the implication of TRPA1 in itch, suggests this condition as a convenient model for the development of therapies targeted to TRPA1-mediated diseases, in particular those featuring pain and neurogenic inflammation. It must be noted, however, that the most popular agonists used so far to induce human TRPA1

activation *in vivo* (AITC and cinnamaldehyde) are far from acting solely on this channel (20, 26, 124, 140, 241, 269, 554, 555, 811), which urgently prompts for the development of more specific agonists.

7.4. TRPA1 IN CARDIOVASCULAR DISEASES

The application of TRPA1 agonists causes dilation of several arteries and these responses are smaller when the vessels are treated with TRPA1 blockers or in preparations isolated from *Trpa1* deficient mice (76, 227, 282, 313, 429, 432, 669, 676, 783).

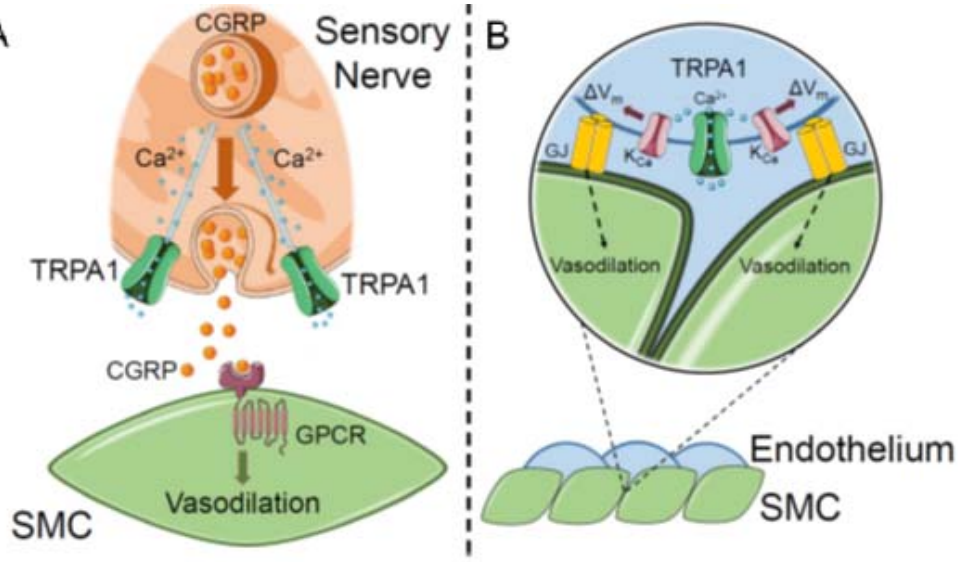

Figure 10: Two proposed mechanisms of TRPA1-dependent vasodilation. (A) The activation of TRPA1 channel in perivascular sensory nerves leads to a Ca^{2+} influx that subsequently causes the release of CGRP from the sensory nerves that innervate the vascular wall. CGRP binds its G protein-coupled receptor (GPCR) on smooth muscle cells (SMC) and causes membrane hyperpolarization, myocyte relaxation and arterial dilation. **(B)** TRPA1 agonists in the blood stream may activate the channel in endothelial cells resulting in Ca^{2+} influx. High intracellular Ca^{2+} leads to hyperpolarization of the endothelial cells via the stimulation of the Ca^{2+}-activated K^+ channels (K_{Ca}). This hyperpolarization is conducted to adjacent SMC, through myo-endothelial gap junctions (GJ), hyperpolarizing the vascular SMC leading to vasodilation.

The peripheral vasodilating effects of the TRPA1 agonist cinnamaldehyde (61) were first reported over 40 years ago (303). This effect is accompanied by a release of catecholamines, suggesting that the effects of this compound are dependent on sensory nerve-mediated mechanisms (302, 347). TRPA1 is expressed in the adventitial nerves in rat femoral, mesenteric and meningeal arteries and in chemoreceptor afferents of chicken aorta (76, 669, 913). Another prominent TRPA1 agonist, allicin (492), is proposed to protect against coronary endothelial dysfunction

via its vasodilatory action on vascular endothelium (62, 459, 786). TRPA1 is also expressed in endothelial cells from mouse and rat cerebral arteries, mouse and rat mesenteric arteries, rat femoral arteries and in rat vascular smooth muscle cells (VSMC) (227, 783, 920). These findings raise the possibility that not only mechanisms mediated by sensory nerves are responsible for TRPA1-dependent vasorelaxation. To date, two mechanisms have been proposed to explain the TRPA1-dependent vasodilation: the nerve-evoked vasodilation and the endothelium-dependent vasodilation (110, 225) (Figure 10).

In this context, it was suggested that TRPA1 activation in sensory nerves induces the release of CGRP, which then binds to its G protein-coupled receptor expressed on the VSMC membrane, causing myocyte hyperpolarization and relaxation (76, 224, 228, 282, 283, 432). Alternatively, the Ca^{2+} influx via TRPA1 activation in endothelial cells may lead to VSMC relaxation and vasodilation (225). In this regard, it is notable that TRPA1 expression is abundant in the endothelial cell plasma membrane that is in proximal contact with VSMC. These junctions host the cellular signaling players necessary for endothelium-dependent VSMC hyperpolarization and vasodilation, such as Ca^{2+}-activated K^+ channels (K_{Ca}) and myo-endothelial gap junctions (291, 454, 711). The activation of TRPA1 (by AITC) in endothelial cells induces Ca^{2+} influx and vasodilation of pressurized rat cranial vessels. AITC-induced vasodilation is abrogated by the inhibition of TRPA1 by HC-030031, the disruption of the endothelium and the inhibition of K_{Ca} channels (227, 291, 676, 783).

Notably, TRPA1-mediated vasodilation in mouse mesenteric artery rings occurs in an endothelial and neuropeptide-independent manner (669). This gave rise to the notion of another TRPA1-induced vasodilation mechanism, presumably working through direct actions on VSMC. However, it is difficult to translate the outcomes of this study since vasodilation was also seen in *Trpa1* KO mice upon administration of

higher doses of cinnamaldehyde. These results suggest that cinnamaldehyde vasodilatory actions may involve TRPA1 independent pathways. In this sense, controversy can also arise from a study reporting an endothelial-independent relaxation of VSMC after stimulation with cinnamaldehyde (920). Moreover, it should be noted that cinnamaldehyde is a potent blocker of the L-type Ca^{2+} channel blocker; expressed in VSMC (26). The reduced Ca^{2+} influx in VSMC, through the inhibition of the L-type Ca^{2+} currents, may partly account for cinnamaldehyde-induced vasodilation.

TRPA1 agonists cause the release of adrenaline from the adrenal cortex (302, 303, 347), which can lead to systemic cardiovascular changes and to a variety of vascular actions in dissimilar vascular beds. A cooperative action of H_2S and NO is proposed to be required for the TRPA1-CGRP signaling pathway and the regulation of the vascular tone (50, 200, 224, 228, 297, 429, 663). Intriguingly, mesenteric arterioles from *Trpa1* KO mice have significantly less ability to relax in response to NO compared to arteries from WT animals (111). Another cooperative mechanism that involves the contribution of TRPA1 in peripheral vasoactivity is the cold-mediated vasoconstriction/vasodilation. This cyclic physiological response to long cold exposures is characterized by a transient initial vasoconstriction due to the activation of sympathetic nerves that is followed by vasodilation mediated by sympathetic nerve inhibition (465, 730). In these responses, TRPA1 and TRPM8 can act as vascular cold sensors mediating mice hind paw vasoconstriction. The cold-induced contractions of the vessels are only prevented by the simultaneous application of TRPA1 and TRPM8 blockers (637, 638). Remarkably, the relaxation of the blood vessel wall is accompanied by increased CGRP and NO levels. More importantly, a TRPA1-TRPM8 synergy does not seem to be necessary in this mechanism since the

vasodilation in response to cold exposures are abolished by the separate use of a TRPA1 or a TRPM8 antagonist (636).

It is suggested that TRPA1-expressing sensory neurons may be involved in the vascular component of neurogenic inflammation. The activation of TRPA1 in sensory fibers by toxic metabolites (such as HNE, N-acetyl-p-benzoquinoneimine and acrolein) caused edema in rat hind paws and tracheal plasma extravasation in mice, rats and guinea pigs. TRPA1 activation leads to a release of pro-inflammatory neuropeptides, such CGRP and substance P (40, 580, 829). The edema and the tracheal plasma extravasation are attenuated by the blockade of TRPA1 and in mice lacking the *Trpa1* gene. Moreover, various TRPA1 agonists may contribute to the formation and propagation of pro-inflammatory factors, for example ROS. In endothelial cells of cerebral arteries, the TRPA1 activation by ROS, and the consequent Ca^{2+} entry, induces dilation of cerebral arteries from wild type but not from *Trpa1* KO mice (783). ROS production is exacerbated in cerebral arteries in pathological conditions such as hypoxia (664) and hypertension (640). For example, hypoxia induces activation of TRPA1 in the cerebral endothelium leading to vasodilation. This effect constitutes an adaptive response that could help reducing the damage in ischemic stroke (664).

ROS-evoked TRPA1 sensitization was shown in another experimental model. Peripheral post-ischemic dysesthesia is an abnormal sensation that is often accompanied by vascular impairment. The ligation of mice hind limbs induces transient ischemia. Reperfusion of the hind limb, by releasing the ligature, elicits dysesthesia-like behaviors manifested as hind paw licking responses in mice. These responses are inhibited by ROS scavengers, by TRPA1 antagonists and by *Trpa1* deficiency. Likewise, intra-plantar injection of H_2O_2 produced similar paw licking responses. These *in vivo* results correlate with *in vitro* findings in human TRPA1-

expressing cells and mouse dorsal root ganglia (DRG) neurons cultures where H_2O_2-evoked TRPA1 responses are increased after hypoxia pre-treatment (712, 750). In this respect, TRPA1 has been proposed to take part also in chronic post-ischemic pain in rat hind paw. Chronic ischemia increases mechanical and cold allodynia in rat paws as well as HNE and TNF-α and the protein levels of TRPA1 (397). Also, in a rat model of femoral artery occlusion (FAO) protein levels in the DRG neurons innervating the femoral arteries (occluded and control) were assessed following 72 h of occlusion. FAO increased the protein levels of TNF-α, PAR2 and TRPA1, particularly in C-fibers. PAR2 activation and AITC produced an increment in TRPA1 currents amplitude in the DRG neurons isolated from occluded femoral arteries rats that was higher than in control femoral arteries. FAO also increases the sympathetic nerve activity in response to AITC, which is attenuated by the blockade of TRPA1 by HC-030031 or by the suppression of TNF-α (910-913). Similar mechanisms are expected during peripheral atherosclerosis. Actually, TRPA1 expression are increased in macrophage-foam cells in mouse atherosclerotic aortas (940).

TRPA1 is functionally expressed throughout the endocardium, myocardium and epicardium of mouse hearts. Its activation by AITC induces intracellular Ca^{2+} transients through the activation of the Ca^{2+}-calmodulin-dependent kinase II. The Ca^{2+} transients are abolished by HC-030031 and absent in *Trpa1* KO mice (42, 43). However, the real physiological contribution of TRPA1 to the cardiac function remains unknown. Nonetheless, an altered TRPA1 activity during pathophysiological conditions could be relevant for the heart, for instance in the cases of ischemia-reperfusion injury, oxidative stress and diabetic cardiomyopathy (171, 488, 619, 877). Recently, a role for TRPA1 in cardiac pathophysiology was related to the exposure to high levels of toxic substances, such as tobacco smoke, diesel exhaust and airborne pollutants. In this sense, gaseous inhalation of high doses of acrolein and ozone

induces changes in heart rate, electrocardiogram, arrhythmias, blood pressure and breathing rate in WT mice. In contrast, neither acrolein nor ozone produced variations of these parameters in *Trpa1* null mice. Accordingly, WT mice treated with HC-030031 were largely protected from acrolein-induced mortality (3, 172, 438, 439). In line with these findings, spontaneous hypertensive rats exposed to diesel exhaust or AITC presented higher sensitivity to trigger arrhythmias. When pre-treated with the TRPA1 antagonist ruthenium red the heightened sensitivity to develop arrhythmic events was prevented (306, 327).

In summary, TRPA1 is involved in the regulation of the vascular tone via its functional expression in the perivascular sensory innervation, in the endothelium and in VSMC. Do to its high sensitivity to endogenous signals released upon tissue damage (e.g., ROS), this channel seems to be a crucial player in ischemia and reperfusion and, together with TRPV1, is a plausible candiate for the initiation of cardiac pain. However, further translational research is needed to determine whether TRPA1 is a potential therapeutic target for the prevention or treatment of cardiovascular diseases.

7.5. TRPA1 IN GASTROINTESTINAL TRACT DISEASES

The enteric nervous system (ENS) is a complex cellular network that consists, among various cell types, of intrinsic and extrinsic afferent nerve fibers. The latter are deriving from nodose ganglia (NG) and DRG that innervate different regions of the gastrointestinal (GI) tract. Extrinsic afferent nerve fibers contribute to the detection of luminal stimuli and convey visceral inputs to the central nervous system (CNS) (202, 246, 261). TRPA1 has been proposed to sense various environmental factors that enter in contact with the digestive system (107, 323, 594). Transcripts and functional expression of TRPA1 have been found in NG and DRG neurons innervating mouse

and rat stomach and colon (128, 417, 822), mouse jejunum and ileum (128, 144, 162, 501, 654) and guinea pig esophagus (482, 931, 932). TRPA1 has been also detected in intestinal tissue samples of mouse, guinea pigs and dog (71, 214, 654), as well as in rat and human colonic epithelial cells (362, 363) and enterochromaffin cells (EC), mouse duodenal and colonic enterocytes (255) and mouse and human mucosa (81, 214, 604, 675). Moreover, TRPA1 is expressed in mouse enteroendocrine cells (EEC), commonly in the duodenum and jejunum, and in human enteric glial cells cultures, where TRPA1 transcripts are upregulated by *E. coli* lipopolysaccharides incubation (159, 476). More importantly, TRPA1 has been found in mouse colonic (but not duodenum, ileum and jejunum) and human intestinal myenteric and motor neurons (29, 527, 668). These enteric neurons, by interacting with other intestinal cell types, control various GI functions, such as motility, epithelial barrier function and ion secretion (138, 261). Therefore, the expression of TRPA1 in intrinsic, neuronal and non-neuronal cells of the intestines may contribute to physiological functions of this channel distinct from its somatosensory role in gut-projecting sensory neurons in normal tissue (108, 128). In fact, a role for TRPA1 in rat and human colonic mucosa ion secretion has been proposed. The application of the channel agonist AITC induces mucosal Cl^- and HCO_3^- secretion in a concentration-dependent manner. The anion secretion in the mucosa layer is abolished by the use of HC-030031. This TRPA1-related anion secretion in response to colonic luminal stimuli is proposed to be mediated by direct activation of the channel or by a prostaglandin E_2 synthesis mechanism independent of neural pathways in the colon (362, 363). TRPA1 is also proposed to contribute to intestinal motility and gastric accommodation responses. TRPA1 agonists accelerate the colonic transit in mouse and may be effective in the treatment of constipation (413). Furthermore, intracolonic treatment of AITC increases colonic motor responses and defecation patterns in conscious dogs. TRPA1-mediated cholinergic and serotonergic

2469 neuronal pathways are important for the AITC-induced colonic motility (in enteric
2470 neurons) and the generation and propagation of giant migrating contractions (in
2471 extrinsic nerves fibers) (214, 709, 756). Similarly, gastric accommodation and
2472 emptying is decreased by treatment with TRPA1 agonists, which increases the
2473 gastric tone via TRPA1-mediated cholinergic and serotonergic neuronal pathways
2474 (215, 423). Additionally, a decreased adrenal sympathetic nerve activity upon gastric
2475 application of β-eudesmol is only observed in WT but not in *Trpa1* KO rats (621,
2476 623). In this context, garlic powder, containing the TRPA1 agonist allicin, induces
2477 gastric relaxation and epigastric symptoms of pressure and warmth in healthy
2478 subjects (257). Nonetheless, the TRPA1 contribution to the regulation of the GI
2479 motility is not restricted to ENS intrinsic and extrinsic afferent pathways. Serotonin (5-
2480 HT) is abundantly stored in EC, which are mucosal endocrine cells present in the GI
2481 tract length. TRPA1 channels in EC not only chemosense the gut environment (81),
2482 but mediate the stimulation of 5-HT release from the EC to the intrinsic and extrinsic
2483 afferents in the ENS. Thus, secreted 5-HT promotes the excitation of neuronal
2484 pathways and, subsequently, physiological and pathophysiological responses (216,
2485 238, 604, 834). It was later concluded, however, that 5-HT does not mediate the
2486 motor effect of AITC in the guinea-pig small intestine (710). The TRPA1 agonist
2487 methylglyoxal induces 5-HT secretion in RIN-14B cells derived from a rat pancreatic
2488 islet tumor (a model for EC cells) and this is reduced by TRPA1 inhibition (789).

2489 TRPA1 has been implicated in digestion and satiety mechanisms. TRPA1 is co-
2490 expressed with cholecystokinin (CCK), an endogenous brain-gut peptide, and 5-HT
2491 in duodenal and jejunal EEC. It was proposed that TRPA1 agonists in garlic,
2492 cinnamon and ginger help digestion by facilitating the CCK and 5-HT secretion from
2493 the EEC in a TRPA1 Ca^{2+} influx-dependent manner (159, 255, 569, 675, 923). On
2494 the other hand, in cranial visceral CCK-expressing nodose neurons, AITC evokes

inward currents that are abolished by HC-030031. Since cranial visceral afferent pathways probably have an effect of satiety, the action of spiced diets, such as garlic and cinnamon, may contribute to reduction of the food intake and be associated with satiety sensations (160). In addition to this, oral treatment with AITC suppresses food intake and increases emesis in mice. These reflexes are abolished by pre-treatment with RR, an unspecific TRP channel blocker, and are proposed to act in a CCK-TRPA1 fashion (819, 947).

The GI tract is continuously exposed to a variety of gut microorganisms that are implicated in several physiological functions such as food digestion, nutrition and immune integrity. Environmental factors, including stress, smoking and diet changes (e.g. pungent compounds in food), can deregulate the immunity system of the GI tract and its epithelial barrier function (17, 81, 324). This unbalanced state leads to chronic inflammatory disorders of the GI tract, i.e., idiopathic inflammatory bowel disease (IBD), ulcerative colitis and Crohn's disease (725, 906). These disorders are characterized by an increased visceral hypersensitivity (VH) due to lower perceptual thresholds for pain and discomfort (852, 893). The mechanisms involving lower GI tract pain and VH are associated to a peripheral sensitization of neuropeptidergic visceral afferent fibers (841, 848). TRPA1 co-localizes with substance P, CGRP, tyrosine kinase A (TrkA) and TRPV1 in the mechanosensitive afferents nerve fibers that projects to the mucosal and serosal/mesenteric layers (128, 144, 417). In this sense, TRPA1 is proposed to contribute to GI inflammation and pain responses mechanisms since its activity in gut afferent fibers regulates the release of neuropeptides during inflammatory conditions (60, 448, 933). Activation and sensitization of TRPA1 in these fibers contributes to the release of substance P and CGRP, which may induce and maintain GI inflammatory states in mice, rats and increase visceral muscle contractions in *D. melanogaster* (60, 92, 239, 887, 916,

933). Notably, it has been shown that TRPA1 has a protective role in a T-cell-mediated colitis model by inhibiting TRPV1 activity in CD4 T-cells (96).

Current experimental methods to study inflammatory states in the GI tract include the exposure of intestine sections to irritant chemicals, such as AITC (392, 530), which lead to experimental IBD. After the IBD is induced, visceral pain is assessed by electromyography (EMG) recordings that estimate the visceromotor response (VMR) to mechanical colorectal distention (CRD) (381, 544). Induced colitis, by administration of trinitrobenzene sulphonic acid (TNBS), provokes a TRPA1-mediated increment in the VMR to CRD and promotes the secretion of substance P and CGRP by colonic DRG neurons (107, 239, 851). Hypersensitive VMR, and the release of substance P and CGRP, are reduced in *Trpa1* KO mice and in mice with reduced TRPA1 expression on the DRG neurons that innervate the colon. Additionally, the TNBS or AITC-induced VH is accompanied by higher TRPA1 mRNA, protein levels and currents in colonic DRG neurons of mouse and rat (144, 162, 239, 392, 418, 468, 501, 560, 922). Moreover, TNBS-induced colonic inflammation in rats increases the H_2O_2 levels and VH. These effects are reverted by HC-030031, suggesting a TRPA1 role in visceral mechanosensation (405). Accordingly, gastric distention induces visceral pain via the activation of extracellular signal-regulated protein kinase 1/2 (ERK1/2) and mitogen-activated protein kinase (MAPK) in the TRPA1-expressing NG and DRG afferents that innervate rat stomach (417, 419). Likewise, mechanosensitivity is increased in mice colonic and guinea pig esophageal sensory afferents upon stimulation of the adenosine A_2 receptor or by the application of the inflammatory mediator bradykinin (128, 131, 482, 931, 932).

Inflammatory agents can directly activate (e.g. HNE) or indirectly sensitize (e.g. agonists of PAR2) TRPA1, thereby inducing pain and hyperalgesia in the GI (144, 239). Histamine-mediated sensitization of TRPA1 most likely contributes to the

2547 increased visceral pain perception in IBS patients (59). Also, the stimulation of PAR2
2548 in mast cells leads to TRPA1 activation and it is proposed as a mechanism for
2549 TRPA1-mediated hyperalgesia (144). PAR2 was found to co-localize with TRPA1 in
2550 CGRP-positive colonic nerve fibers in an inflammatory TNBS rat model (147).
2551 However, *Trpa1*-null mice show no changes in mechanosensory functions after
2552 PAR2 activation (128). Although TRPA1 is suggested to be involved in the
2553 pathogenesis of IBD, its role in nociception during inflammation (i.e. the involvement
2554 of PAR2) remains controversial. TRPA1 is upregulated in inflamed human and
2555 mouse colon samples and has protective roles through the reduction of the

2556 expression of multiple pro-inflammatory neuropeptides, cytokines and chemokines
2557 (431). In the same way, novel TRPA1 activators, such as carvacrol, carvacry and
2558 ASP7663, reduce the production of pro-inflammatory cytokines and ROS in a mouse
2559 jejunal mucosal inflammation model and the VMR to CRD in rat constipation and pain
2560 model (23, 24, 414). Of note, the downstream factors of TRPA1 pathways, substance
2561 P and CGRP release, play opposite roles in IBD. Substance P is implicated in the
2562 pathogenesis of IBD and CGRP is proposed to exert a protective role in colitis (239,
2563 841). Furthermore, *Trpa1* KO mice are resistant to experimental colitis, have no
2564 differences compared to control mice, or present more prominent inflammatory

extents in the intestines (144, 239). Several factors, including species differences, diversity of genetic backgrounds and variations on the GI tract regions and their corresponding innervations (e.g. colon-splanchnic nerve or esophagus-vagal nerve) may explain the disparity of these results (112, 239). In spite of this, TRPA1 is considered a potential target in the treatment of inflammatory pain and mechanosensitivity in GI disorders (60, 80, 953) (Figure 11).

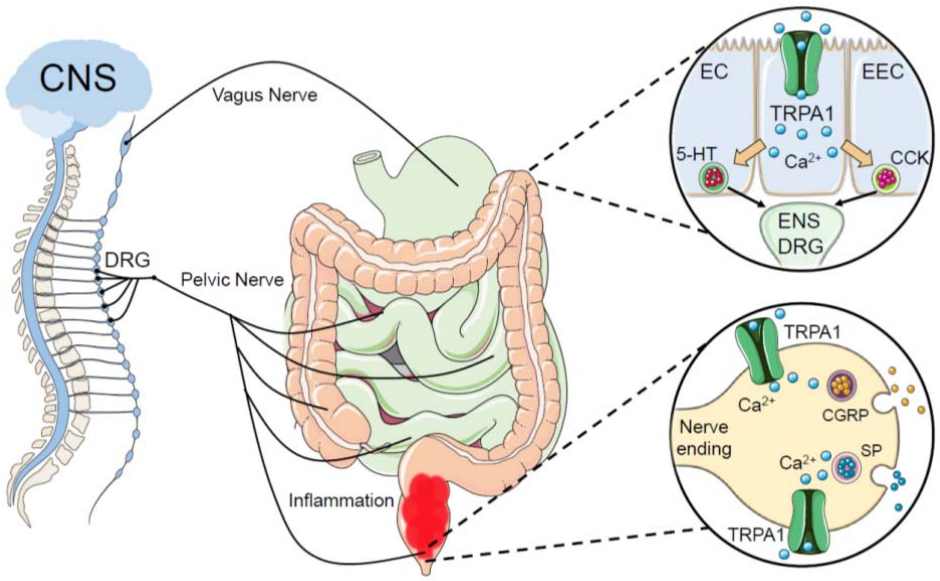

Figure 11: TRPA1 agonists may modulation gastrointestinal motility. Following its activation in enterochromaffin cells (EC) and enteroendocrine cells (EEC), the TRPA1-induced intracellular Ca^{2+} increase gives rise to increased secretion of serotonin (5-HT) and cholecystokinin (CCK), which eventually trigger activity of the myenteric and DRG neurons embedded in the myenteric plexus. In addition, the TRPA1-expressing sensory nerves in the GI tract (via Ca^{2+} influxes) mediate the release of neuropeptides (such as substance P; SP and calcitonin gene-related peptide; CGRP) in response to GI

Stressful life events can have striking influences on visceral perception in IBS patients. TRPA1 is involved in the stress-induced visceral hyperalgesia in rats

probably due to an upregulation of the channel in the colonic afferent DRG. This suggests that TRPA1 could be a target to treat the stress-induced visceral hyperalgesia in IBS (934). Interestingly, a novel TRPA1 variant (c.2755C>T), found in a parent-son pair, selectively co-segregated with cramp-fasciculation syndrome symptoms such as IBS and GI refluxes (598).

In summary, it is clear that TRPA1 plays pathophysiological roles in the GI tract. Yet, a better understanding its contributions to gut related syndromes is required in order to treat patients with GI complaints.

7.6. TRPA1 IN DIABETES, OBESITY AND PANCREATITIS

Diabetes mellitus is the most common metabolic syndrome in humans and it is characterized by increased blood glucose concentrations. Type I diabetes occurs in response to idiopathic or immune-mediated destruction of pancreatic β-cells that results in a failure of the pancreas to secrete enough insulin. On the other hand, type II diabetes (T2DM) is due to resistance to insulin in the target tissues. Nonetheless, as T2DM progresses it is common that a dysfunction of the pancreatic β-cells follows the pre-existent condition leading to hypoinsulinemia rather than a peripheral insulin resistance (592, 658, 957). The TRPA1 agonist cinnamaldehyde has been long used in traditional medicine as anti-diabetic (6, 592, 658, 755). However, the mechanism underlying the effects of this compound on lowering hyperglycemia is presumably TRPA1-independent. TRPA1 is functionally expressed in EEC in primary mouse intestinal cultures. Agonists of the channel promote the glucagon-like peptide-1 (GLP-1) secretion, a blood glucose-lowering hormone, from EEC (237, 947). The GLP-1 secretion, however, is not observed in primary intestinal cultures derived from *Trpa1*-null mice (947). TRPA1 is expressed in rat pancreatic β-cells where, upon application of its agonist (like HNE or AITC), contributes to increase intracellular Ca^{2+}

levels leading to insulin release. On the contrary, the TRPA1 antagonist HC-030031 inhibits the glucose-induced insulin release suggesting a possible role for this channel in the release of insulin (139, 607).

Current therapies for diabetic patients include the oral treatment with sulfonylureas, such as glibenclamide. Sulfonylureas block K_{ATP} channels, increasing the insulin release from pancreatic β-cells. Despite of this, sulfonylureas are ineffective for long-term treatments and have associated undesirable effects (607, 658). In pancreatic β-cells, sulfonylureas can activate TRPA1, which can potentially contribute to the sulfonylureas-induced toxicity due to the cooperative insulinotropic effects that results from both the inhibition of K_{ATP} channels and the activation of TRPA1 (209).

Diabetes is a chronic disease that aggravates with time. A complication of diabetes is the peripheral diabetic neuropathy (PDN). In this scenario, the TRPA1 activation in small diameter fiber endings is proposed as a mechanism that contribute to PND in the early phase of diabetes (411). Diabetes induces endoplasmic reticulum (ER) stress. This condition can be experimentally modeled by tunicamycin, which produces ER stress and neuropathic pain in rats (343). In addition to ER stress, the hyperglycemic conditions stimulate the release of a cocktail of TRPA1 agonist (such as HNE, methylglyoxal and ROS) causing mechanical allodynia in diabetic rodents. Both ER stress-induced and TRPA1-induced mechanical hypersensitivity are attenuated by the use of TRPA1 blockers and in *Trpa1* KO mice (33, 70, 230, 288, 312, 336, 343, 409, 411, 490, 624, 684, 879, 880). In line with these findings, TRPA1 blockers delay the loss of substance P immunoreactivity-expressing small diameter bra endings in diabetic animals (409). Curiously, TRPA1 is involved in PND sensitivity to cold but not in the mechanical hyposensitivity in later phases of diabetes (318). It should be mentioned, though, that streptozotocin (STZ) directly activates

TRPA1 (31). Thus, PND models should be redefined, because the direct potentiation of TRPA1 by STZ complicates the interpretation of some of the previous cited results.

Apart from PND, diabetes also carries other life-threatening problems, for example cardiovascular complications. In human cardiac fibroblasts, methylglyoxal induces a Ca^{2+} influx that is inhibited by HC-030031. The use of siRNA to knock down TRPA1 in cardiac fibroblasts reduces the methylglyoxal-evoked Ca^{2+} entry (619). This study suggests that a TRPA1-mediated mechanism is involved in the development of diabetic cardiomyopathy, which represents a major cause of morbidity and mortality in T2DM.

Yet, obesity is, undoubtedly, the most common risk factor for this metabolic disease (658). As mentioned above, TRPA1 is proposed to play a role in food intake and satiety (159, 160, 569, 675, 819, 903, 904, 923, 947). Thus, TRPA1 has been considered as a therapeutic target for appetite suppression in obesity (957). In this context, some flavonoids (from citrus origin and with therapeutic value in the prevention and treatment of cardiovascular diseases (27, 296)) have been used as TRPA1 activators to stimulate the release of CCK in STC-1 cells (an EEC cell line model). The flavonoids naringin and hesperidin, as well as their aglycones naringenin and hesperetin, respectively, increase intracellular Ca^{2+} via TRPA1 potentiation. Therefore, a dose-dependent stimulated CCK secretion is observed, which could contribute to appetite regulation and food intake (390, 643). A similar approach is proposed with cinnamaldehyde, which by triggering TRPA1 activity in EEC reduces the levels of secreted ghrelin, “the hunger hormone” (137, 951). Furthermore, in an adipocyte cell line, TRPA1 activation reduces lipid accumulation (471). Interestingly, TRPA1 agonists induce adrenaline secretion via CNS, which prevents fat accumulation and obesity in mouse (347, 505, 625, 804, 878). Altogether, this

evidence suggests that these natural compounds are candidate biomolecules for satiety control in obesity in T2DM.

Chronic pancreatitis (CP) is a devastating inflammatory disease that exacerbates over time and leads to permanent damage of the pancreas. It is characterized by persistent abdominal pain and ultimately impairs the ability to digest food and the production of pancreatic hormones. Pancreatic inflammation involves central and peripheral sensitization (551). Pancreatic peripheral innervation consists of NG and DRG sensory neurons projections that are likely to innervate the duodenum (466, 720). TRPA1 channels, expressed in these neurons, are involved in pancreatic inflammation by sensing the physical and chemical injuries in the pancreas. As in GI inflammation models, TNBS is also used to induce CP in mice. The inflammation and pain induced in this model are significantly lower in *Trpa1* KO mice (143). In a more common CP mouse model, induced by cerulein, an increment in DRG TRPA1 immunoreactive fibers and transcripts is observed. TRPA1 antagonists, or the deletion of *Trpa1* attenuates the cerulein-induced pancreatic inflammation (146, 720, 721). Moreover, inflammatory mediators that activate TRPA1 (like HNE, PGE2 or H_2S) increase pancreatic inflammation and pain in these mice (146, 812). Additionally, the TRPA1 activation mediates PAR2 stimulation, which contributes to pancreatic nociceptor excitation (813). The evidence suggests that TRPA1 contributes to sensing pancreatic inflammation, although a synergistic interaction of TRPA1 with other proteins, such as TRPV channels is needed (146, 551, 720, 721). A novel TRPA1 and TRPV blocker inhibites inflammation and pain-associated behavior in a mouse model of acute pancreatitis (368). Consequently, TRPA1 is an interesting drug target for the treatment of CP, although more profound investigations are required.

7.7. TRPA1 IN RESPIRATORY DISEASE

TRPA1 plays a fundamental role not only for the normal airway function (556), but becomes particularly important for respiratory diseases characterized by hypersensitivity, for instance, asthma, rhinitis, chronic obstructive pulmonary disease (COPD) and chronic cough (272, 273, 484, 863, 921). Chemosensory airway reflexes can provoke severe complications in patients affected by inflammatory airway conditions (899).

The whole respiratory tract is innervated by primary sensory afferent nerves, which can be activated by mechanical and chemical stimuli via activation of TRPA1 on these vagal sensory afferents (85, 326, 564, 577, 777). TRPA1 is expressed in the nasal mucosa (582) and it is primarily expressed in small-diameter nociceptive neurons, where its activation contributes to the perception of noxious stimuli in the airways (686). TRPA1 expression is found also in immune cells, smooth muscle cells, and airway epithelial cells (133, 369, 561, 564, 584, 824). Airway sensory nerves may respond via TRPA1 to exogenous (AITC, cigarette smoke, chlorine, tear gas) and endogenous irritants (40, 41, 99, 130, 383, 928). TRPA1 activation in the nasal cavity induces significant bradypnea and a decrease of the respiratory frequency (344). Nasal trigeminal nerve endings are particularly sensitive to oxidants formed in polluted air and during oxidative stress as well as to chlorine, which is frequently released in industrial and domestic accidents (97, 98, 100).

Cough is a defense mechanism mediated by the vagus nerve that protects the airway by clearing the respiratory tract of potentially harmful irritants and pathogens. Broncho-pulmonary vagal fibers respond to a variety of TRPA1 agonists (132, 562, 577, 686) and trigger cough in animals and human (106, 805). Paroxysms of coughing are worsened by numerous TRPA1 agonists in chronic cough patients

(557). For example, recent data demonstrates an interaction between diesel exhaust particles (DEP), which can contain electrophilic and non-electrophilic TRPA1 agonists (194) and airway C-fiber activation. Components of DEP lead to mitochondrial ROS production, which is known to activate TRPA1 on nociceptive C-fibers (686, 805). In addition, TRPA1 is activated by compounds found in wood/biomass smoke particulate materials (195). TRPA1 antagonists or the anti-oxidant N-acetylcysteine attenuate IL-8 expression in human lung cells exposed to combustion particles (350). Also, compounds within cigarette smoke such as acrolein and crotonaldehyde cause cough through activation of TRPA1 (75, 76, 106, 727), suggesting a role for TRPA1 in cigarette smoke-induced cough, which is a key disease driver of COPD. TRPA1 is activated by the chemical sensitizer toluene diisocyanate, a potent sensory irritant known to cause chemical-induced occupational asthma (206). TRPA1 is also involved in the induction of mouse lower airway hyperreactivity to methacholine by exposure to chlorine and ovalbumin, an experimental non-allergic model that may be relevant for cleaning workers and competitive swimmers (331).

The airways of asthmatic patients contain increased levels of PGE2 and bradykinin, which activate TRPA1 and TRPV1 and trigger coughing (280, 497). However, activation of TRPA1 inhibits the airway smooth muscle cell proliferative phenotype, a key contributing factor to asthma (937). A strong association was found between TRPA1 gene variants, childhood asthma and total IgE concentrations (263).

Nasal hyperreactivity is a common feature in patients suffering from allergic and non-allergic rhinitis (846). These symptoms have been postulated to arise from hypersensitivity of the trigeminal fibers innervating the nasal mucosa (274). Recently, it was shown that patients suffering rhinitis exhibit a decreased threshold to the TRPA1 agonist AITC (845, 846), which correlates strongly with total symptom scores

and is resolved after chemical destruction of the nasal sensory nerves (845, 846). Repeated applications of azelastine hydrochloride and fluticasone propionate (MP29-02) mouse sensory neurons expressing the transient receptor potential channels TRPA1 and TRPV1 (422). This effect may contribute to the therapeutic action (reduction of inflammatory mediators and nasal hyperreactivity) of this formulation in allergic rhinitis.

TRPA1 is expressed in non-neuronal cells in the respiratory tract such as human lung fibroblast cells and pulmonary alveolar epithelial cells. Activation of TRPA1 potentially induces the release of chemokines in inflamed airways (561), and conversily, inflammatory cytokines can enhance TRPA1 translocation to the membrane (791). An *in vitro* model of respiratory virus infection of human bronchial epithelial cells showed that cytokines released in the supernatants lead to increased TRPA1 expression (631). The TRPA1-mediated responses to cigarette smoke may result, not only from the activation of the channel in sensory nerve endings but also in airway epithelial cells (474, 868). This can be secondary to ROS production and resulting in IL-8 production via the activation of the MAPKs/NF-κB pathway (474). Additionally, the exposure to cigarette smoke extract increase TRPA1 expression in airway epithelial cells (590). In HBECs, TRPA1-dependent Ca^{2+} influx following cigarette smoke exposure is secondary to ROS production and result in IL-8 production via the activation of the MAPKs/NF-κB pathway. Nevertheless, a study on high-level acrolein-induced toxicity suggests that TRPA1 plays an important protective role in both acute and post-exposure processes (172). The pseudostratified columnar epithelium of the bronchial lumen in cystic fibrosis co-expresses IL-8 and TRPA1. Here, the inhibition of TRPA1 expression results in a relevant reduction of release of several cytokines, including IL-8 and the proinflammatory cytokines IL-1β and TNF-α. This suggests that TRPA1 mediates the

severity of airway inflammation driven by cystic fibrosis bronchial epithelial cells (672). A nasal challenge with AITC induces the release of the mucin 5 subtype (MUC5B) a mucin specifically associated with inflammatory airway disease (16).

Thus, the lungs and in particular the upper airways constitute a primary target of environmental pollutants that can activate TRPA1 and thereby induce well-known protective reflexes, such as mucus secretion, sneezing and cough. The ventilatory function of the airways can be assessed by monitoring ventilatory parameters recorded with plethysmography. As up until now, e.g., (100, 801), future studies can employ this relatively simple and non-invasive technique to continue decyphering TRPA1 airway pathophysiology and therapeutics. A particularly intriguing issue is to determine the implication of TRPA1 in the induction and maintenance of airway hypersensitivity conditions, especially as a sensor of thermal changes and chemical irritants, which are still known as unspecific environmental stimuli in the clinical research community. Other advances in this direction may be achieved with the use of nasal mucosal potential measurements (403, 524, 816). This methodology allows monitoring the responses of sensory nerves to chemical stimuli in humans, in a rather non-invasive and simple manner and has already served to test the effects of AITC in human nasal mucosa in pathological conditions (845).

7.8. TRPA1 IN THE UROGENITAL SYSTEM

The lower urinary tract (LUT) contains the bladder and urethra, which are involved in the involuntary storage and voluntary expulsion of urine. Overactive bladder (OAB) is a common LUT pathology associated to detrusor muscle overactivity. In OAB the properties of bladder afferent pathways are affected, leading to bladder storage dysfunctions and, subsequently, to an increase in urinary urgency, frequency and pain (36, 747). TRPA1 is proposed to contribute to the bladder function since TRPA1

proteins and transcripts are found on mechanosensory lumbosacral fibers that innervate mouse, rat, guinea pig and human urinary bladder mucosa (urothelial and sub-urothelial spaces), pig ureter and human urethra (169, 221, 249, 284, 528, 564, 589, 778). TRPA1 mRNA and proteins are found in the bladder wall, particularly, in rat and human urothelial cells and rat and human SMC. TRPA1 is also found in blood vessels throughout rat bladder, pig and human interstitial cells and human prostate (221, 242, 284, 359, 430, 768, 778, 885).

TRPA1 may play an important role in the regulation of bladder contraction. Agonists of TRPA1 (AITC and CA) increase the bladder contraction, stretch sensitivity, hyperalgesia and micturition in rats and guinea pigs (37, 193, 222, 589). Moreover, in various OAB models, TRPA1 protein and mRNA expression levels are increased in the bladder and in the DRG neurons innervating it (38, 193, 221, 376, 499, 539, 634, 862). This upregulated TRPA1 expression may increase sensory transductions and induce the OAB symptoms. Furthermore, the TRPA1 antagonist HC-030031 diminished OAB symptoms and decreased the micturition reflexes (38, 156, 222, 522). In cyclophosphamide-induced OAB models, the toxic metabolite and TRPA1 agonist acrolein is produced. Acrolein can be responsible for the bladder inflammatory responses in cyclophosphamide-induced cystitis through direct activation of the channel (249, 271, 525). Additionally, in the rat model PAR2 expression is enhanced. The blockade of PAR2 reduces the TRPA1 signaling pathway and attenuates OAB symptoms (149). Similarly, another compound produced in inflammatory states, nitro-oleic acid (OA-NO_2) enhances the contractile activity of rat bladder strips by TRPA1 activation in afferent nerves (45). In the same way, the administration of H_2S, a bacterial metabolite synthesized during inflammation, induces urodynamic parameters changes, presumably via TRPA1 activation in the DRG neurons innervating the bladder (467, 525, 645, 778). TRPA1

co-expresses with TRPV1, substance P and CGRP in the bladder nerve endings (51, 222, 284, 589, 778), hence the notion of TRPA1 as an important player in inflammatory bladder (cystitis) conditions. Bladder contractions and pain-like behavior in response to TRPA1 agonists (or inflammatory mediators, e.g. LPS) are accompanied by increased levels of substance P, CGRP and PGE2 (37, 359, 364, 659, 886). This suggests that the TRPA1-mediated contraction of the detrusor muscle involves the stimulation and secretion of neuropeptides and prostanoids from sensory afferents (747). Remarkably, TRPA1 agonists have no effect in spontaneous contractions of isolated human urethral strips (284, 886). Conversely, after phenylephrine-induced contraction, TRPA1 agonists induce a dose-dependent relaxation of urethral strips, probably in a TRPV1-dependent mechanism (284, 886). However, this does not exclude that TRPA1 is involved in the initiation of afferent activity in pathophysiological states (36, 364). It should be noted, though, that the role of TRPA1 in the LUT has been mostly assessed using agonists and antagonists of the channel. Some studies should be carefully interpreted since the TRPA1 agonist AITC can also activate TRPV1 (241). On the other hand, only two studies involved *Trpa1* KO mice, but showed a reduced number of voids and pain-like behaviors in *Trpa1* KO mice compared to WT after ROS- or LPS-induced cystitis (634).

The data indicates that TRPA1 contributes to urinary bladder function in disease. This organ is another very good model to further investigate the pathophysiological roles of TRPA1. Indeed, the use of cystometry (842), allows tracking robust reflexes of the bladder wall upon intravesical administration of chemical irritants in anesthetized or awake animals, and can therefore be used in the development of novel TRPA1 antagonists. Furthermore, voiding reflexes triggered by stimulation of other parts of the body (for instance in the skin) can be used to dissect the roles of

sensory TRP channels (e.g., TRPM8 vs. TRPA1) in the detection of environmental stimuli such as cold (843).

8. CONCLUSIONS

TRPA1, primarily a chemosensor channel with more recently established roles in thermosensation and mechanosensation, is an extremely interesting ion channel, not only for its importance as drug target, but also for its unique structural and biophysical properties. About seven years ago we pinpointed a set of issues that were required to be clarified for a better understanding of the function of this intriguing channel (594). Although immense advances have been made, especially with the appearance of the first high-resolution models of the channel structure (651), we realize that the main challenges remain virtually the same. For instance, we still have very little knowledge about how the interaction of electrophilic agonists with nucleophilic amino acid residues translates into channel opening. Similarly, the mechanisms of Ca^{2+}-dependent potentiation and inactivation, the activation by non-electrophilic compounds and modulation by cold, heat, reactive species and many others factors, remain to be fully elucidated. On the other hand, it is becoming clear that noxious TRPA1 agonists activate the channel via their specific chemical properties, such as electrophylicity, oxidative power, or the ability to acidify the intracellular milieu, rather than by specific molecular structures. This underscores the idea that TRPA1 functions, not as a refined sensor, but as a broadly-tuned detector of almost every potentially injurious external stimulus and damage-associated endogenous signal. Thus, TRPA1 seems to be a channel that when activated informs the sensory systems that something goes wrong, with the mere functions of

triggering immediate protective behavioral responses and putting in motion the machinery of tissue repair.

Data obtained in multiple animal models of disease strongly indicate that TRPA1 is implicated in pain and inflammation, and that it plays important roles in the initiation, progression and maintenance of chronic inflammatory diseases and tissue injury, including asthma, diabetes, arthritis and skin diseases. This prompts for the investigation of cell- and tissue-specific TRPA1 properties that could be of help in the design of specific treatments. It is imperative to notice that the basic knowledge on TRPA1 biophysics, pharmacology and regulation is required to understand the complex pathophysiology of TRPA1, and is particularly essential for the rational design of modulators that may be used as therapeutic agents. For instance, it is important to distinguish between possible beneficial effects due to channel block versus beneficial effects of channel activation. This is because depending on its extent and rate of occurrence, TRPA1 activation may lead to cell excitation or to quiescence via slow depolarization and consequent inactivation of voltage-gated Na^+ and Ca^{2+} channels. The discovery of the functional expression of TRPA1 beyond sensory neurons, i.e., epithelial and smooth muscle cells, fibroblasts, oligodendrocytes, enteroendocrine cells, etc., in which the channel's function is still poorly understood, represents a complication for the development of therapeutic strategies targeting this channel. TRPA1 seems to behave as a molecular sensor with a multi-dimensional operating point, being tightly regulated by pH, oxidation, O_2 levels, electrophiles and nucleophiles, hydroxylation, temperature, membrane lipids, voltage, intracellular Ca^{2+}, trace heavy metals, etc. Thus, it is likely that every cell type in which it is functionally expressed provides a unique scenario for yet unknown amazing features of this intriguingly irritating channel.

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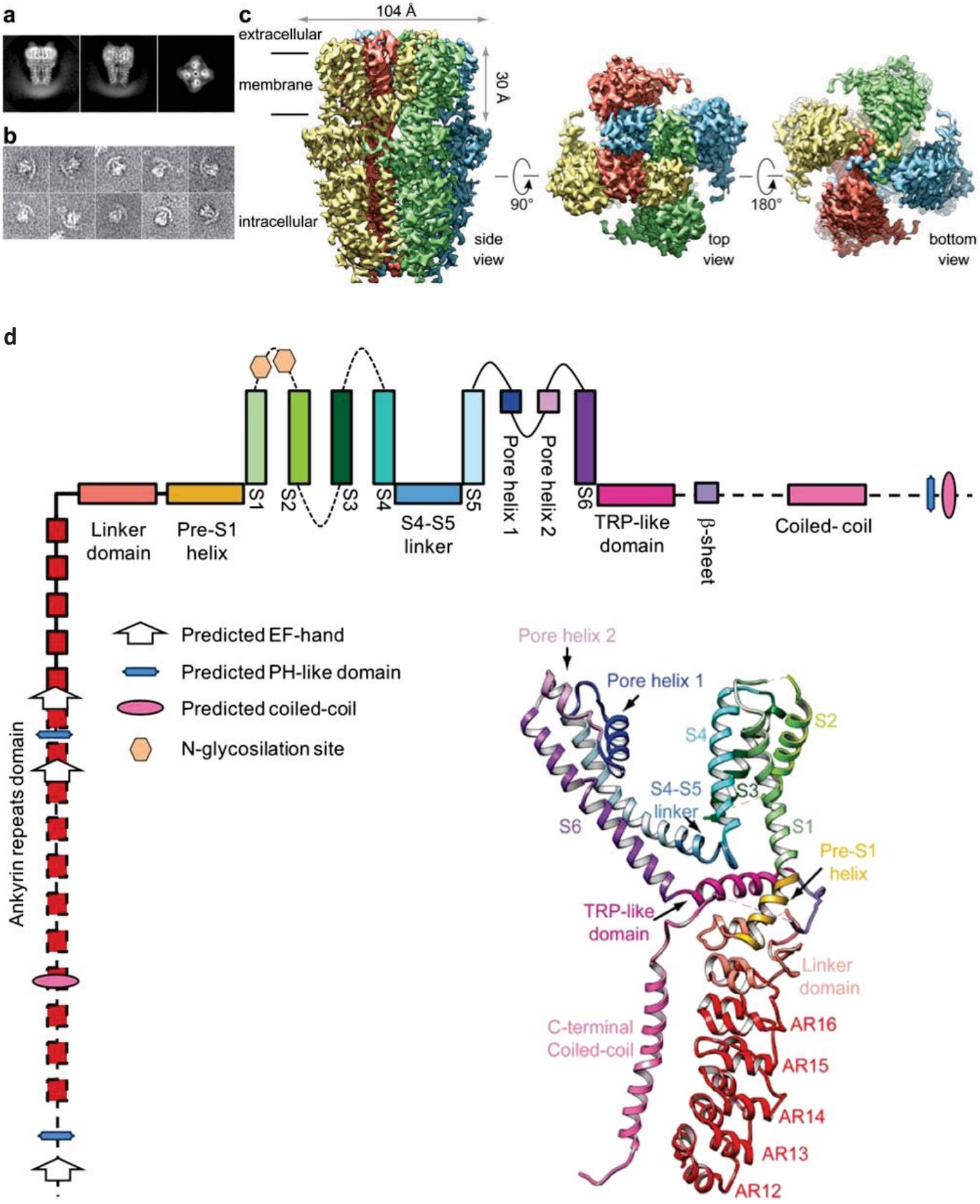

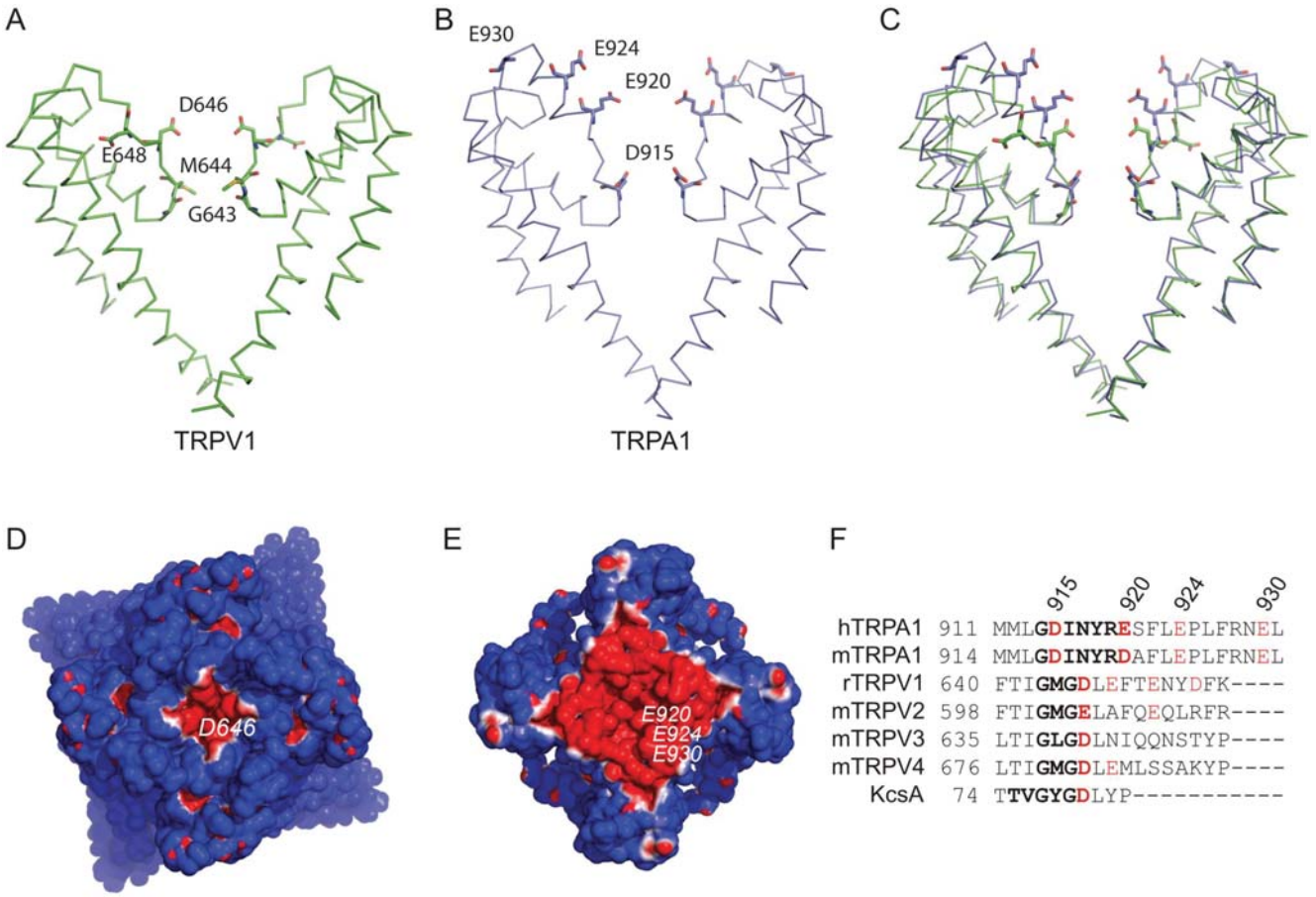

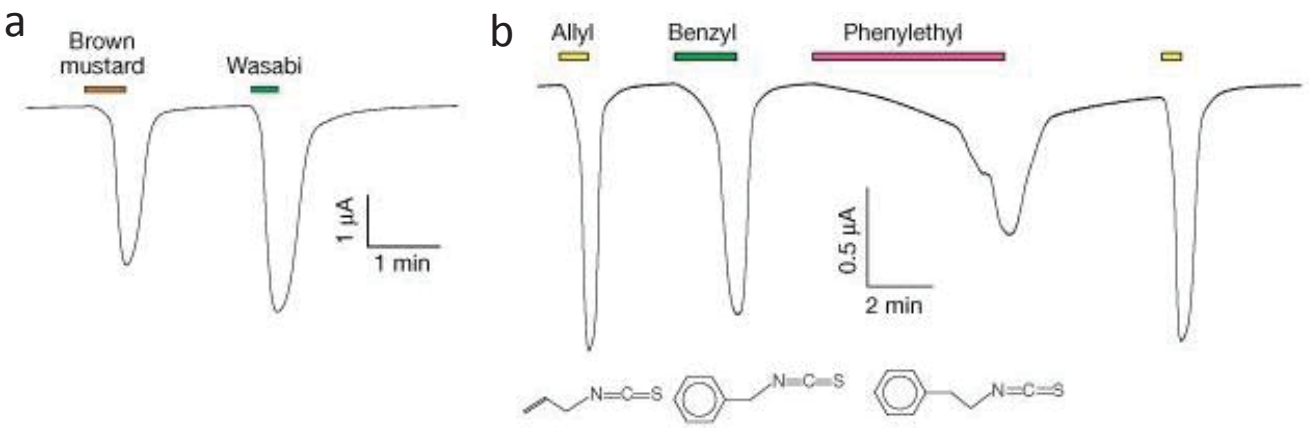

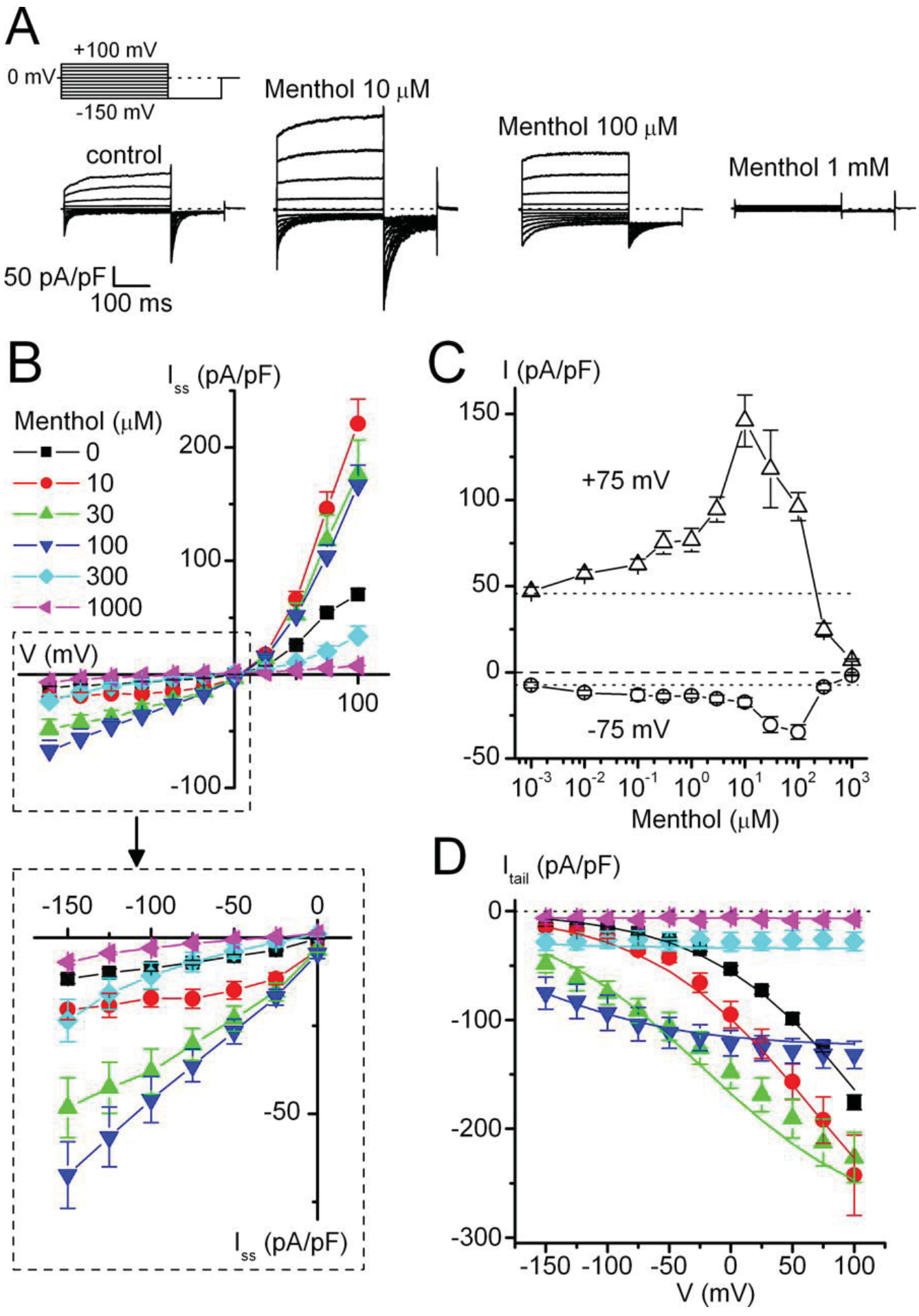

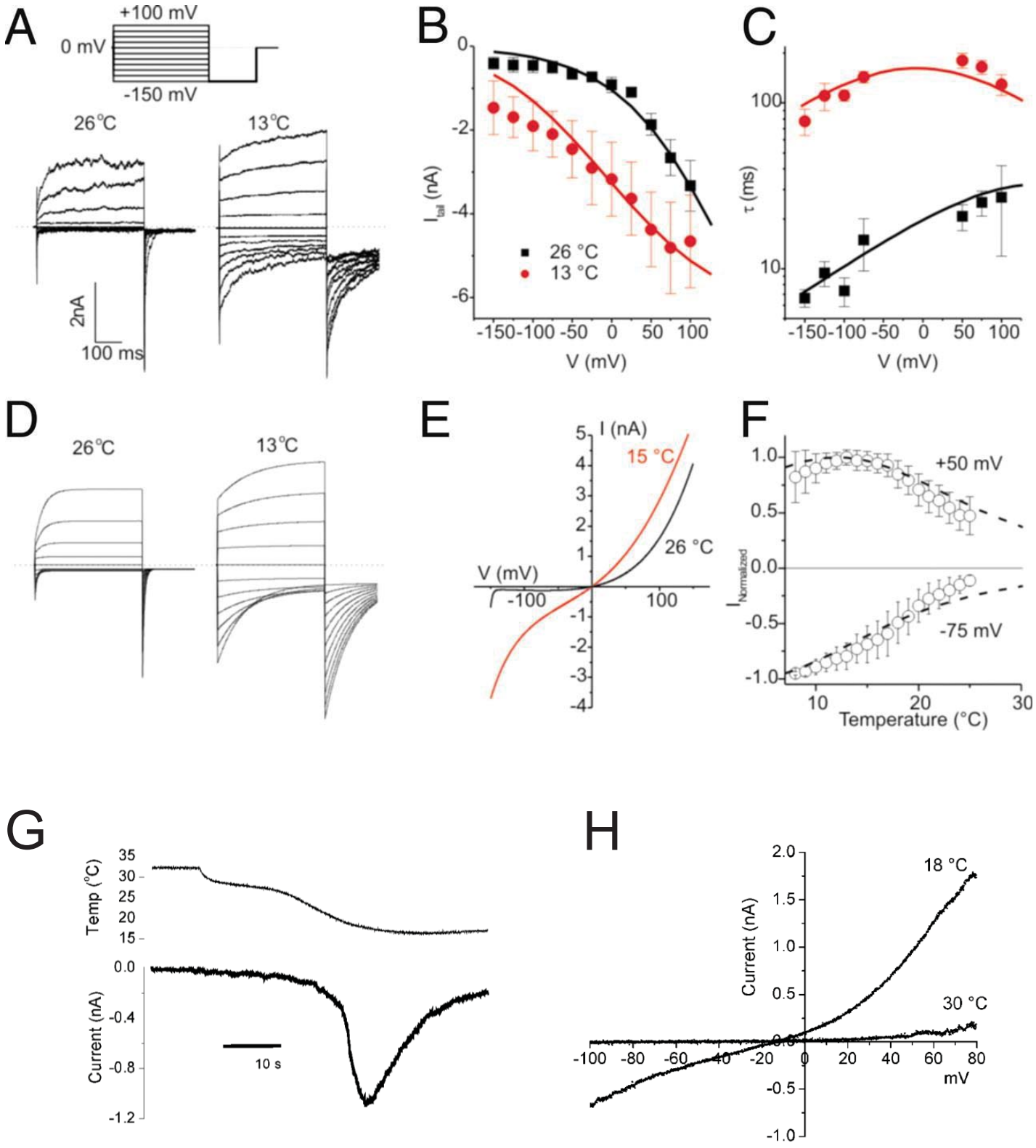

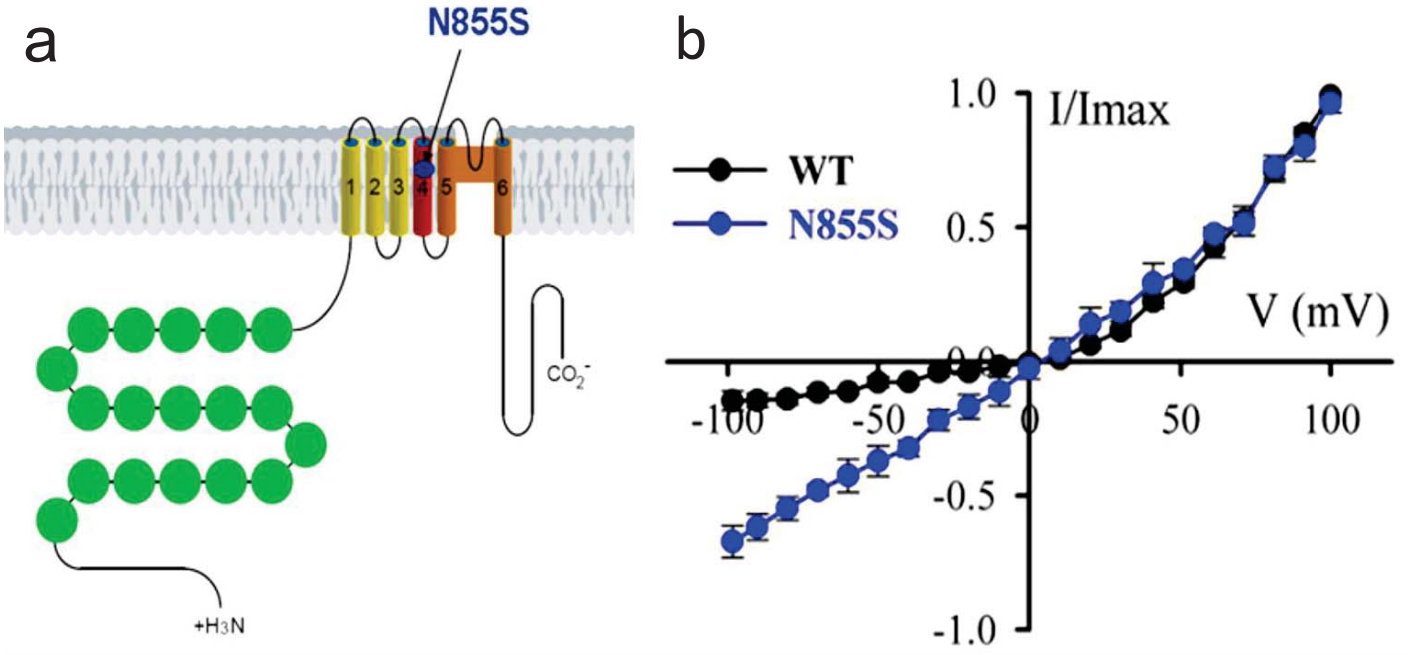

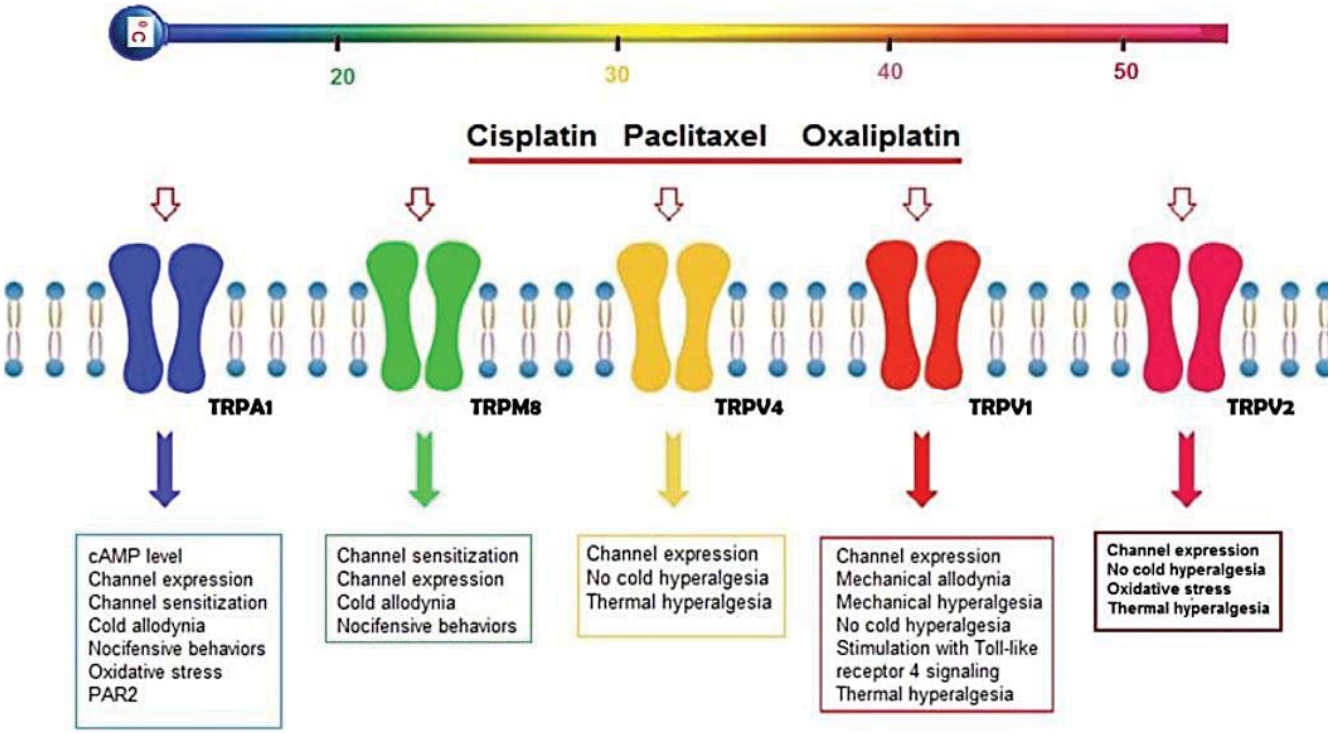

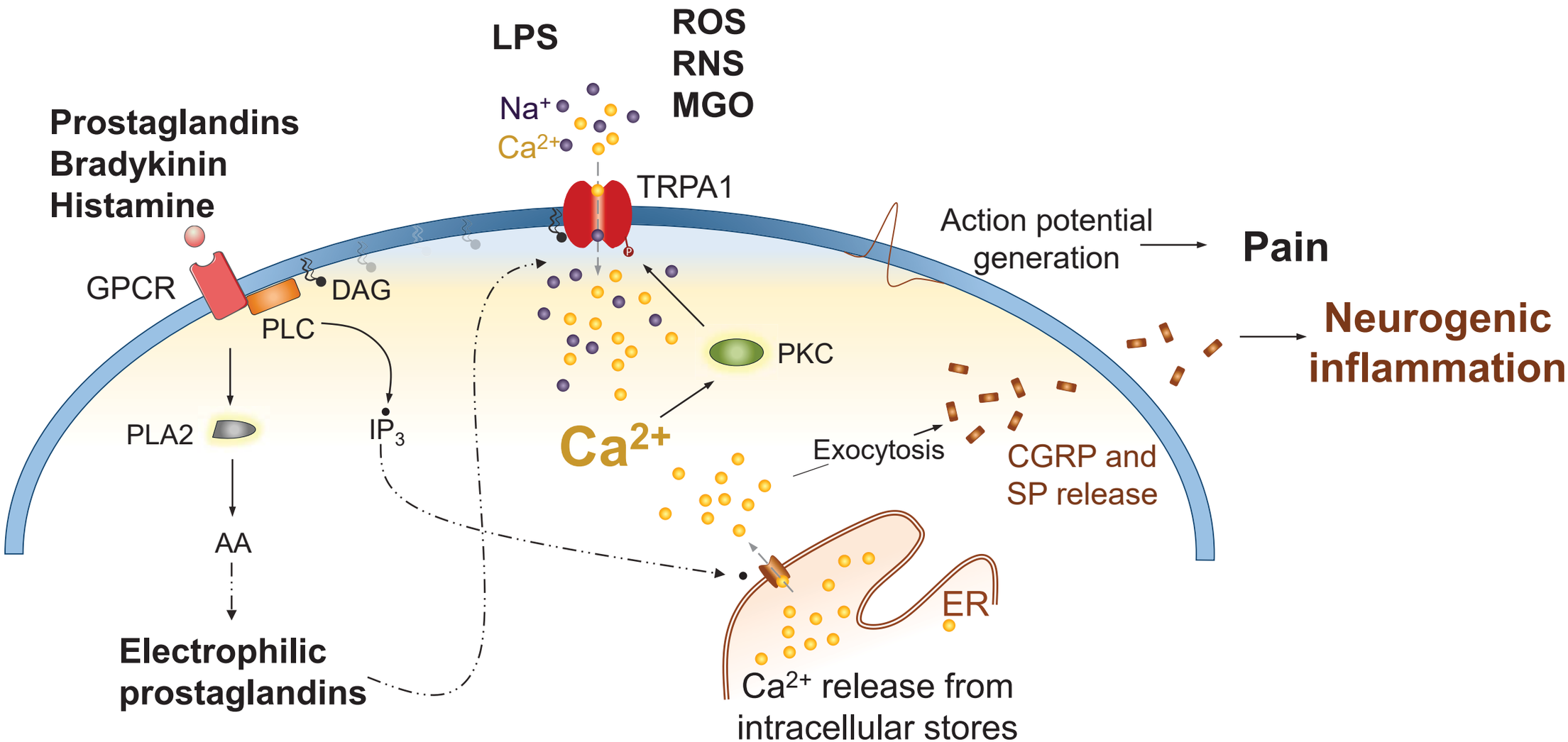

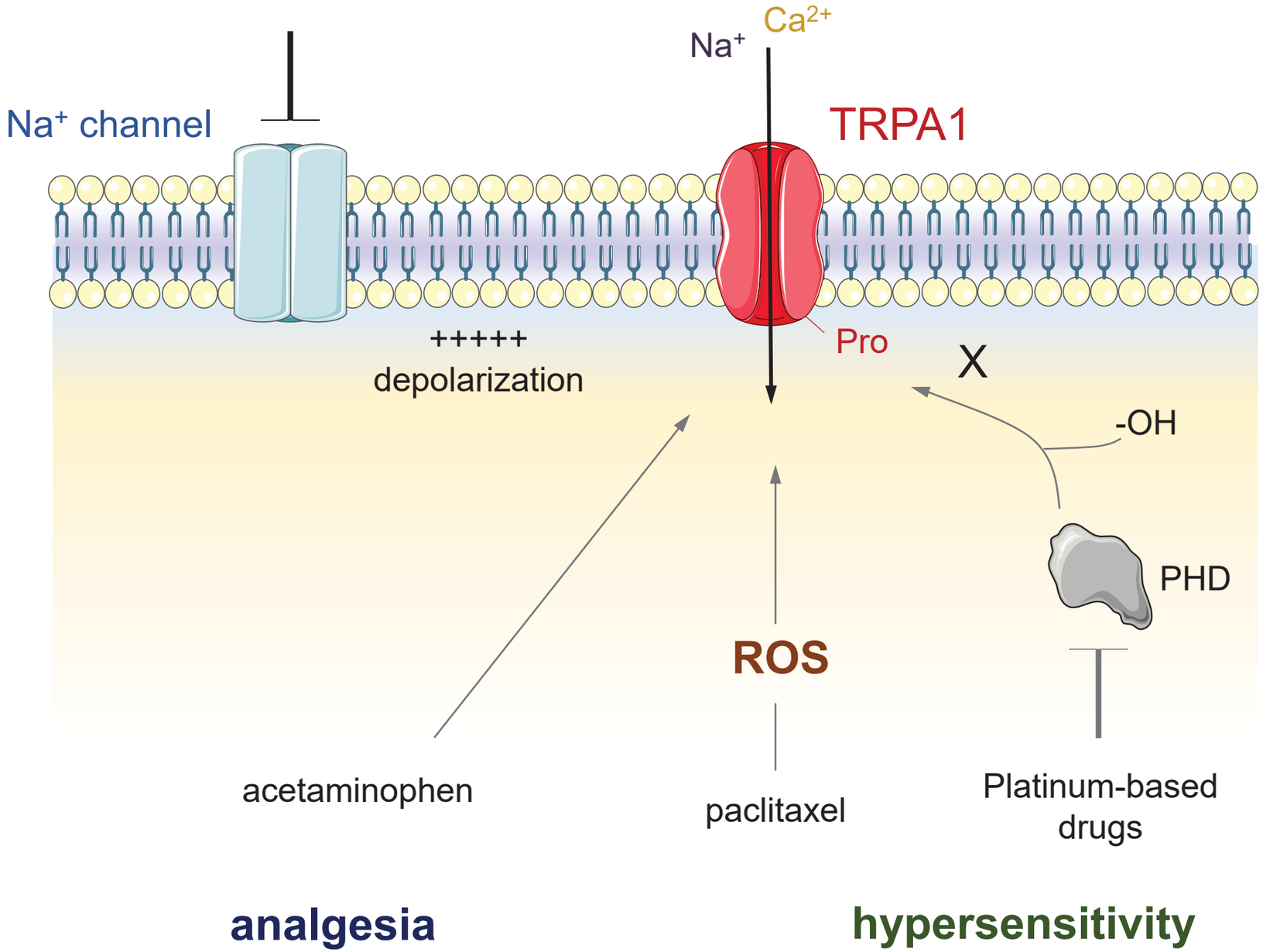

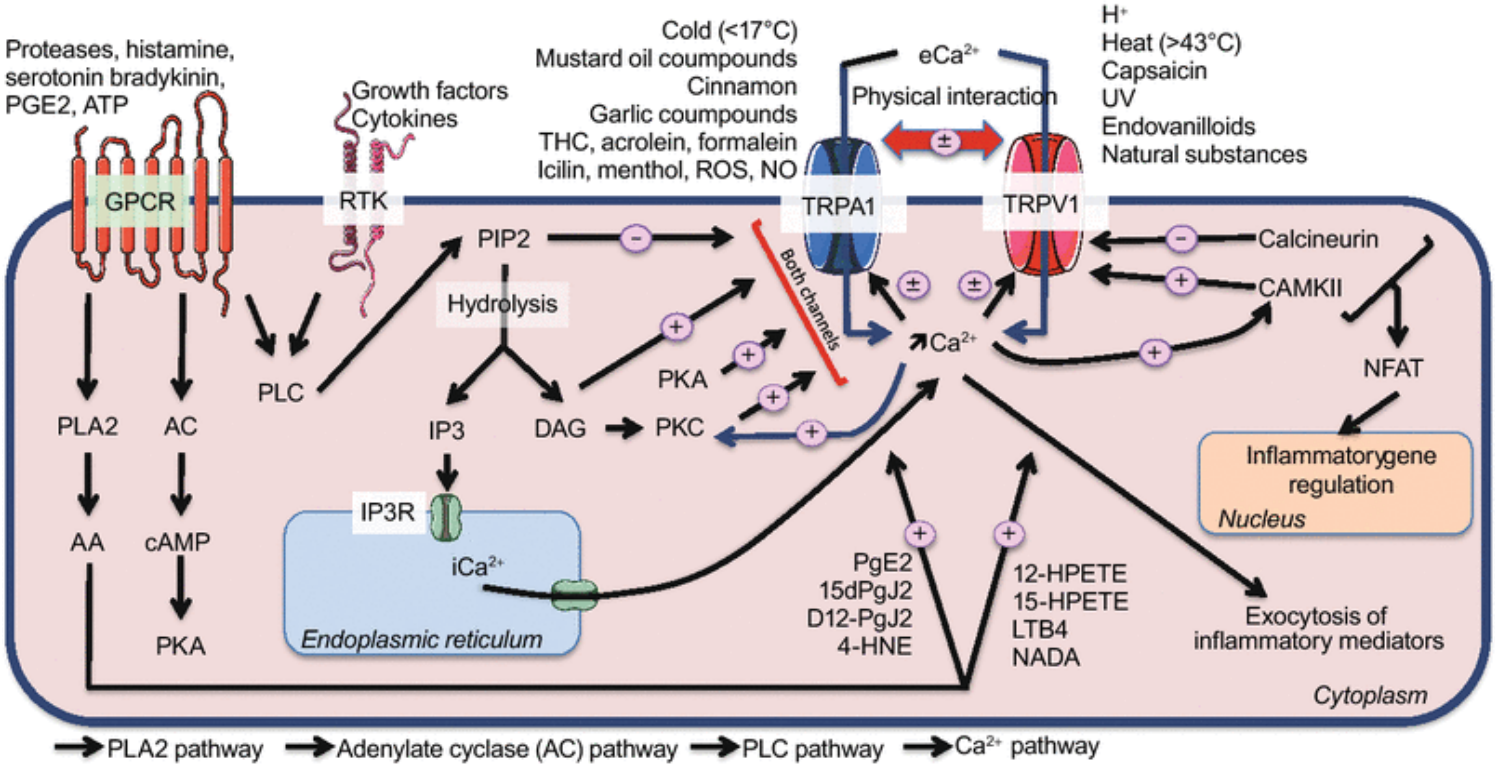

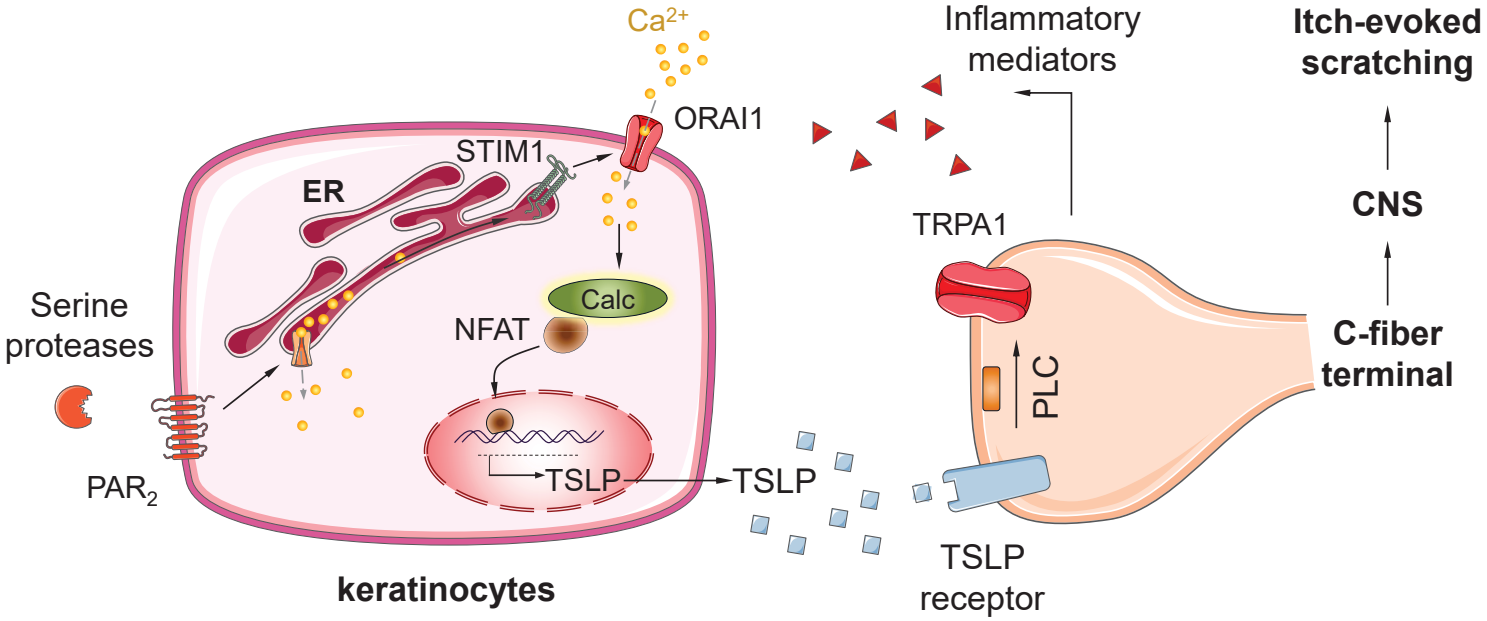

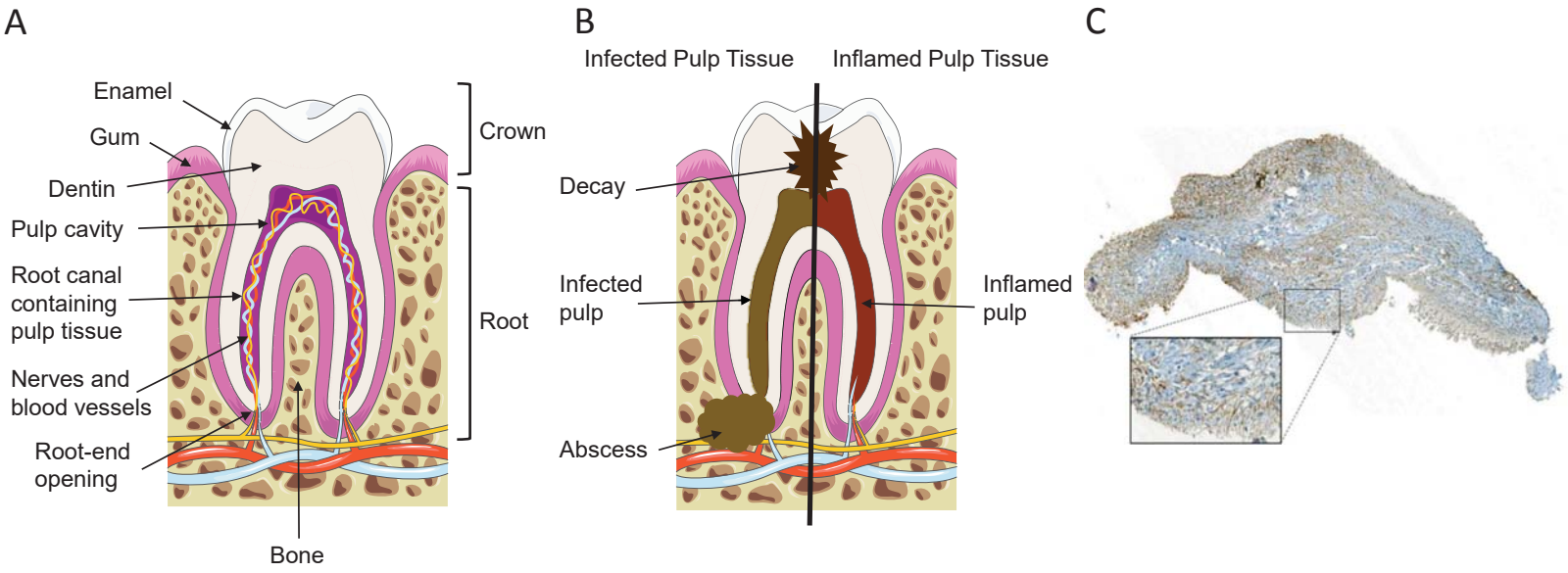

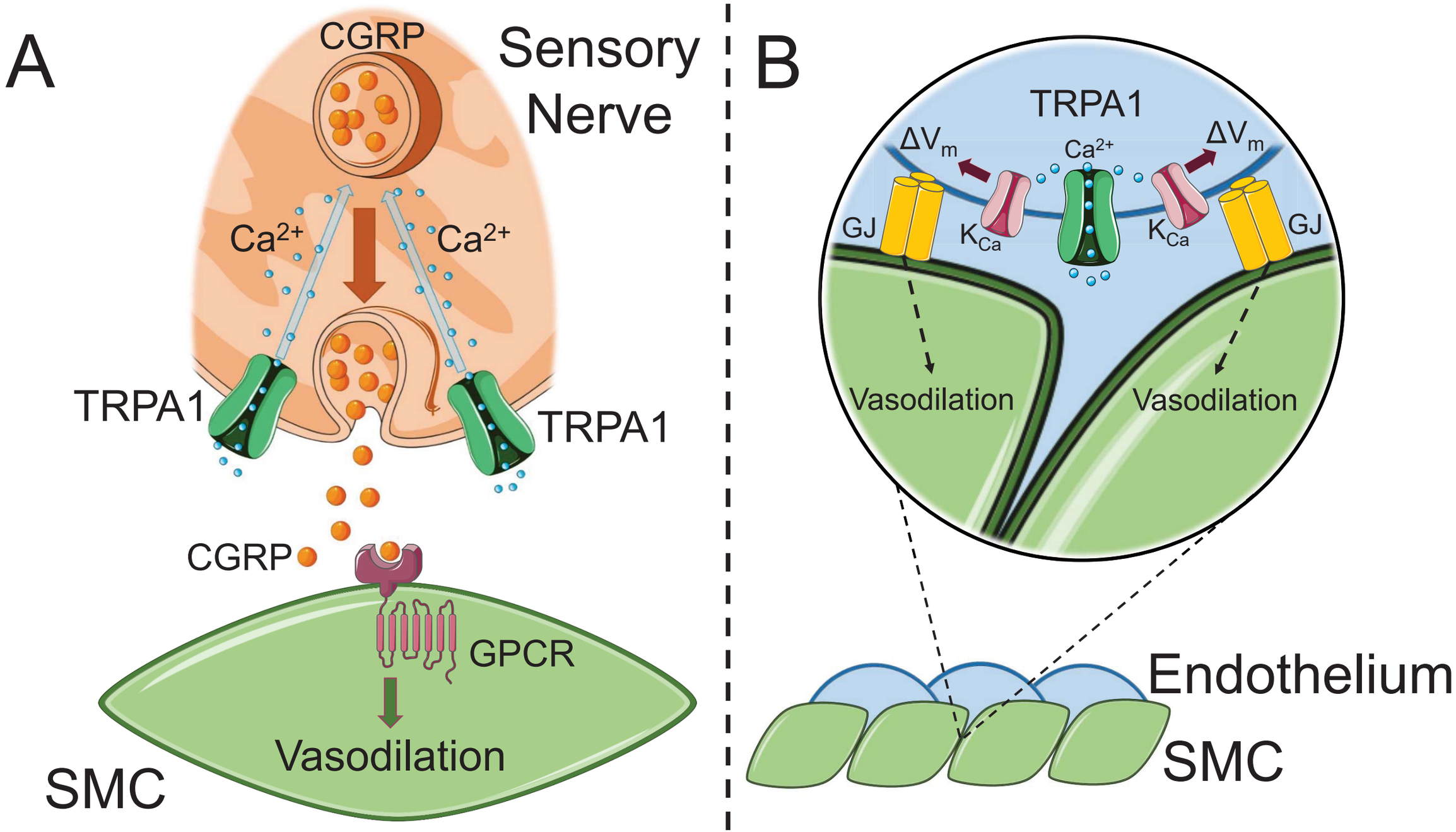

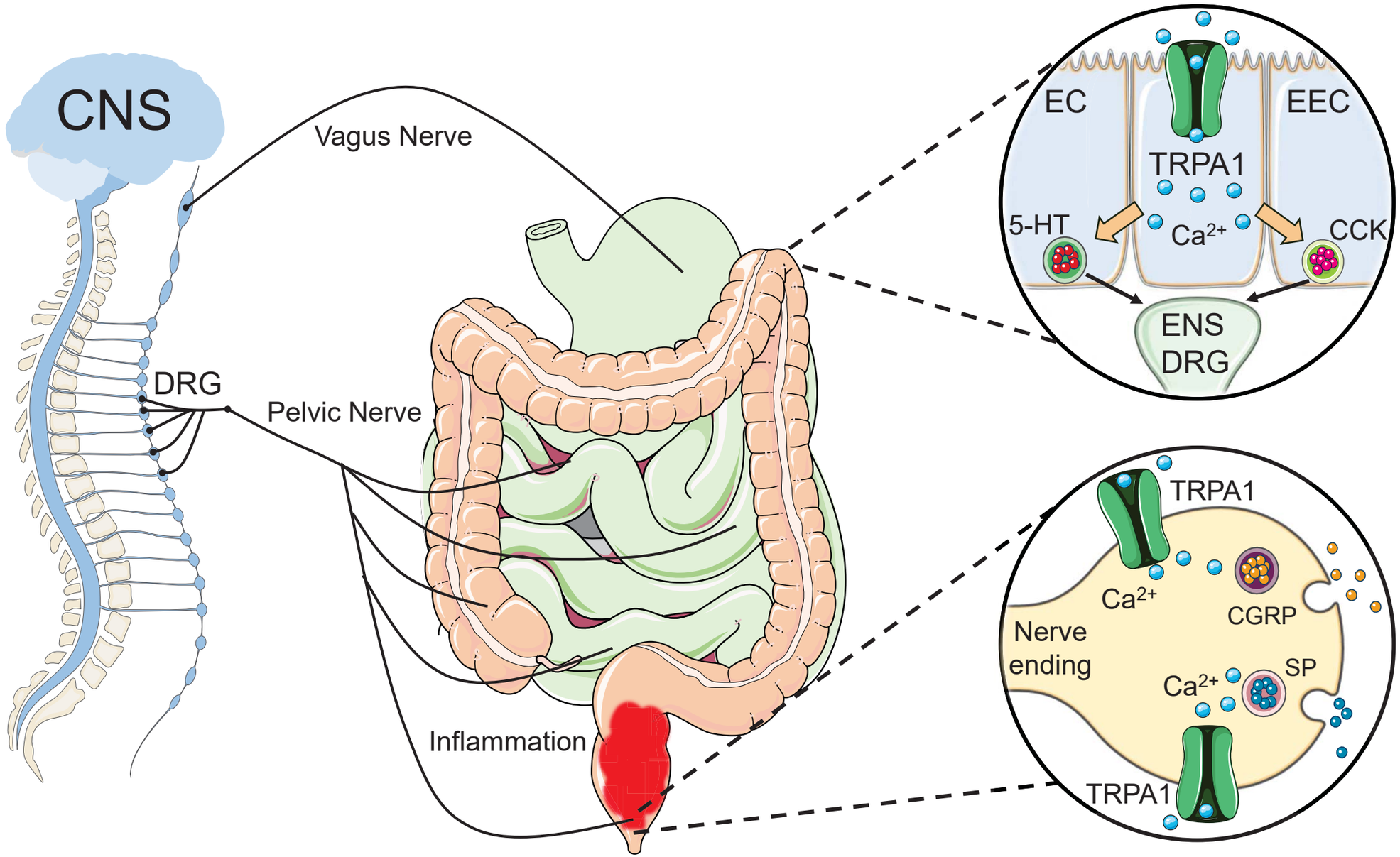

Table 1: Mammalian TRPA1

Common name	Binomial name	GeneID	Chromosome (map location)	GNAv	Exon count
Human	*Homo sapiens*	8989	8 (q21.11)	NC_000008.11	29
Common chimpanzee	*Pan troglodytes*	464230	8	NC_036887.1	27
Sumatran orangutan	*Pongo abelii*	100460642	8	NC_036911.1	27
Western gorilla	*Gorilla gorilla*	101150478	8	NC_018432.2	27
Crab-eating macaque	*Macaca fascicularis*	102139057	8	NC_022279.1	28
Rhesus macaque	*Macaca mulatta*	694623	8	NC_027900.1	27
Northern white-cheeked gibbon	*Nomascus leucogenys*	100607287	16	NC_019831.1	27
Common marmoset	*Callithrix jacchus*	100414472	16	NC_013911.1	27
Bonobo	*Pan paniscus*	100973158	8	NC_027876.1	27
Olive baboon	*Papio anubis*	101016452	8	NC_018159.2	27
Gelada baboon	*Theropithecus gelada*	112630413	8	NC_037676.1	27
House mouse	*Mus musculus*	277328	1 (A3)	NC_000067.6	27
Gairdner's shrewmouse	*Mus pahari*	110338816	22	NC_034611.1	27
Ryukyu mouse	*Mus caroli*	110298275	1	NC_034570.1	27
Common rat	*Rattus norvegicus*	312896	5(q11)	NC_005104.4	27
Prairie vole	*Microtus ochrogaster*	101984403	LG5	NC_022031.1	27
Domestic dog	*Canis lupus familiaris*	486994	29	NC_006611.3	27
Domestic cat	*Felis catus*	101080611	F2	NC_018740.3	27
Domestic goat	*Capra hircus*	102170065	14	NC_030821.1	27
Domestic sheep	*Ovis aries*	101115717	9	NC_019466.2	29
Cattle	*Bos taurus*	505317	14	NC_037341.1	27
Horse	*Equus caballus*	100061564	9	NC_009152.3	27
Przewalski's horse	*Equus przewalskii*	103548063	Un	NW_007673276.1	26
European rabbit	*Oryctolagus cuniculus*	100341337	3	NC_013671.1	27
Wild boar	*Sus scrofa*	100152934	4	NC_010446.5	29
Water buffalo	*Bubalus bubalis*	102397027	15	NC_037559.1	27
Tibetan antelope	*Pantholops hodgsonii*	102315761	Un	NW_005812652.1	28
Polar bear	*Ursus maritimus*	103681282	Un	NW_007927247.1	28
Weddell seal	*Leptonychotes weddellii*	102730954	Un	NW_006383700.1	27
Minke whale	*Balaenoptera acutorostrata scammoni*	103012702	Un	NW_006728019.1	28
Cape golden mole	*Chrysochloris asiatica*	102826219	Un	NW_006408554.1	29
Aardvark	*Orycteropus afer afer*	103202460	Un	NW_006921768.1	27
Cape elephant shrew	*Elephantulus edwardii*	102862466	Un	NW_006399758.1	27

Gray short-tailed opossum	*Monodelphis domestica*	100028386	3	NC_008803.1	29
Tasmanian devil	*Sarcophilus harrisii*	100918272	2	N/A	Unk
Sunda flying lemur	*Galeopterus variegatus*	103585496	Un	NW_007726355.1	27
Big brown bat	*Eptesicus fuscus*	103293988	Un	NW_007370710.1	27

GNAv: genomic nucleotide accession version

Table 2: TRPA1 in non-mammalian species

	Common name	Binomial name	GeneID	Chromosome (map location)	GNAv	Exon count
birds	Red junglefowl	*Gallus gallus*	420180	2	NC_006089.5	27
	Eurasian blue tit	*Cyanistes caeruleus*	111924651	2	N/A	
	Japanese quail	*Coturnix japonica*	107310278	2	NC_029517.1	27
	Great tit	*Parus major*	107214741	2	NC_031769.1	27
	Anna's hummingbird	*Calypte anna*	103527146	Un	NW_007619513.1	27
	Collared flycatcher	*Ficedula albicollis*	101813018	2	NC_021673.1	28
	Zebra finch	*Taeniopygia guttata*	100221097	2	NC_011465.1	27
	Domesticated turkey	*Meleagris gallopavo*	100545876	3	NC_015013.2	14
	Helmeted guineafowl	*Numida meleagris*	110394756	2	NC_034410.1	29
reptile	Green anole	*Anolis carolinensis*	100556580	4	NC_014779.1	30
	Green sea turtle	*Chelonia mydas*	102944221	Un	NW_006642402.1	29
amph	Western clawed frog	*Xenopus tropicalis*	100158526	6	NC_030682.1	27
	African clawed frog	*Xenopus laevis*	108695342	6S	NC_030735.1	27
fish	Japanese rice fish	*Oryzias latipes*	101174541	20	NC_019878.2	28
	Northern pike	*Esox lucius*	105019660	LG21	NC_025988.3	28
	Turquoise killifish	*Nothobranchius furzeri*	107382917	sgr08	NC_029656.1	27
	Mexican tetra	*Astyanax mexicanus*	103042231	3	NC_035899.1	28
	goldfish	*Carassius auratus*	113042317	24	NC_039266.1	32
	Eastern happy	*Astatotilapia calliptera*	113028962	9	NC_039310.1	31
	Atlantic salmon	*Salmo salar*	106579725	ssa19	NC_027318.1	26
	Guppy	*Poecilia reticulata*	103456670	LG20	NC_024350.1	29
	Tongue sole	*Cynoglossus semilaevis*	103377016	3	NC_024309.1	31
	Australian ghostshark	*Callorhinchus milii*	103174784	Un	NW_006890060.1	27
	Spotted gar	*Lepisosteus oculatus*	102688457	LG9	NC_023187.1	30
	Southern platyfish	*Xiphophorus maculatus*	102223701	21	NC_036463.1	29
	Japanese puffer	*Takifugu rubripes*	101075823	10	NC_018899.1	28
	Nile tilapia	*Oreochromis niloticus*	100701720	LG9	NC_031974.2	30

	Zebrafish	*Danio rerio*	474351	2	NC_007113.7	28
insect	Red flour beetle	*Tribolium castaneum*	658860	LG3	NC_007418.3	19
	Common fruit fly	*Drosophila melanogaster*	39015	3L(3-27cM)	NT_037436.4	19
nem	Round worm	*Caenorhabditis elegans*	178118	IV	NC_003282.8	11

GNAv: genomic nucleotide accession version

Compound name	Functionality	EC50 (μM)	IC50 (μM)	Method used	References
allyl isothiocyanate (AITC)	bimodal	64 ± 3 11 ± 1 22 ± 3	 4100 ± 800	hTRPA1 electrophysiology in oocytes (+80 mV) rTRPA1 electrophysiology in oocytes (-60 mV) mTRPA1-expressing CHO cells using FLIPR mTRPA1 electrophysiology in CHO cells (-75 mV)	(265) (304) (54) (200)
cinnamaldehyde (CA)	bimodal	61 ± 9 250 ± 150 400 ± 40	 3500 ± 300	mTRPA1-expressing CHO cells using FLIPR mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging	(54) (16) (282)
super cinnamaldehyde (SCA)	agonist	0.8		using FLIPR	(417)
allicin	agonist	1.3 51 1.9 7.5 ± 0.4		mTRPA1 rTRPA1 hTRPA1-expressing CHO cells using FLIPR hTRPA1 electrophysiology in oocytes (-80 mV)	(418) (67)
diallyl disulfide (DADS)	agonist	192 ± 3 7.6		hTRPA1 electrophysiology in oocytes (-80 mV) hTRPA1-expressing CHO T-Rex using Flex Station II	(67) (353)
diallyl sulfide (DAS)	agonist	254		hTRPA1-expressing CHO T-Rex using Flex Station II	(353)
diallyl trisulfide (DATS)	agonist	0.49		hTRPA1-expressing CHO T-Rex using Flex Station II	(353)

acrolein	agonist	5 ± 1 85 ± 9 0.8		hTRPA1 electrophysiology in oocytes (-60 mV) hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(66) (282) (33)
2-chlorobenzylidene malononitrile (CS)	agonist	0.0009 0.214 0.0007		hTRPA1-expressing HEK293-T-Rex cells using FDSS hTRPA1 electrophysiology in HEK293-T-Rex cells (-30 mV) hTRPA1-expressing HEK293-T-Rex cells using FLIPR	(108) (526)
dibenz[b,f][1,4]oxazepine (CR)	agonist	0.0003 0.063		hTRPA1-expressing HEK293-T-Rex cells using FDSS hTRPA1 electrophysiology in HEK293-T-Rex cells (-30 mV)	(108)
1-chloroacetophenone (CN)	agonist	0.03 0.275		hTRPA1-expressing HEK293-T-Rex cells using FDSS hTRPA1 electrophysiology in HEK293-T-Rex cells (-30 mV)	(108)
ethyl bromoacetate (EBA)	agonist	0.039		hTRPA1-expressing HEK293-T-Rex cells using FDSS	(108)
bromobenzyl cyanide (BBC)	agonist	0.01		hTRPA1-expressing HEK293-T-Rex cells using FDSS	(108)
camphor	bimodal	 ≤ 300	660	rTRPA1 electrophysiology in HEK293 cells (–80 mV) mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(778) (16)
Δ9 tetra-hydrocannabinol (Δ9-THC)	agonist	12 ± 2 0.23 ± 0.03		rTRPA1 electrophysiology in oocytes (-60 mV) rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(304) (157)

plumbagin	agonist	0.46 ± 0.05		hTRPA1-expressing HEK293 cells using intracellular Ca^{2+} imaging	(262)
boropinal A	agonist	10 ± 3		hTRPA1-expressing HEK293 cells using intracellular Ca^{2+} imaging	(262)
juglone	agonist	1.7 ± 0.5		hTRPA1-expressing HEK293 cells using intracellular Ca^{2+} imaging	(262)
nicotine	bimodal	17	4000	mTRPA1 electrophysiology in HEK293 cells (–75 mV)	(682)
4-hydroxyhexenal (4-HHE)	agonist	40 ± 12		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(28)
4-Hydroxy-2-nonenal (4-ONE)	agonist	1.9 ± 0.7		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(28)
4-hydroxynonenal (4-HNE)	agonist	20 ± 3 13 27 9.9 ± 1.2 6.6 ± 1.5 6.0 ± 0.8		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging and FLIPR mTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging hTRPA1 mTRPA1 rTRPA1-expressing HEKT cells using FLIPR	(28) (420) (704) (90)
15-deoxy-Δ12,14-prostaglandin J2 (15d-PGJ_2)	agonist	5.6 ± 1.1 40 ± 16 60 ± 20 5.4 ± 1.1		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging hTRPA1 mTRPA1 rTRPA1-expressing HEKT cells using FLIPR	(28) (90)

hydrogen peroxide (H_2O_2)	agonist	1200 ± 400 (after 90 s exposure) 230 (after 600 s exposure) 290 ± 90 297 ± 9		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging mTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(28) (88) (614)
chloramine-T (N-chloro-sodium-p-toluenesulphenamide)	agonist	11 ± 1		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(88)
formaldehyde	agonist	357 0.0016 ± 0.0001%		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging and FLIPR hTRPA1 rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(420) (440)
hypochlorite (OCl^-)	agonist	11 ± 1 ppm 7 ± 1 ppm		hTRPA1 mTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(88)
icilin	agonist	Above 25			(581, 665)
ozone (O_3)	agonist	3		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(690)
toluene diisocyanate (TDI)	agonist	10000		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(688)
2-chloro-N-(4-(4-methoxyphenyl)thiazol-2-yl)-N-(3-methoxypropyl)-acetamide (JT010)	agonist	0.00065 0.047		mTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(680) (257)
p-benzoquinone (pBQN)	agonist	0.36 ± 0.02 0.44 ± 0.02		mTRPA1 hTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(25)

		3.2 ± 0.6		mTRPA1 electrophysiology in CHO cells (–60 mV)	
N-acetyl-p-benzoquinoneimine (NAPQI)	agonist	0.9 ± 0.3 1.33 ± 0.04		mTRPA1 hTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(25)
Methyl p-hydroxybenzoate	agonist	4400		mTRPA1 electrophysiology in HEKT cells (–60 mV)	(217)
3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597)	agonist	24 ± 3 70 ± 8		hTRPA1 rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(511)
nifedipine	agonist	157 ± 8 140 ± 20 0.40 ± 0.02		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(282) (204)
flufenamic acid (FFA)	agonist	24 ± 3 57 ± 5 44 ± 11 55 ± 4		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging hTRPA1 electrophysiology in WI-38 fibroblasts cells (+100 mV) hTRPA1 electrophysiology in WI-38 fibroblasts cells (-100 mV)	(282)
niflumic acid	agonist	28 ± 3		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging	(282)
mefenamic acid	agonist	61 ± 5		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging	(282)
diclofenac	agonist	210 ± 20		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging	(282)

flurbiprofen	agonist	342 ± 6 310 ± 70		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(282)
indomethacin	agonist	470 ± 50		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging	(282)
ketoprofen	agonist	> 500		hTRPA1 in WI-38 fibroblasts using intracellular Ca^{2+} imaging	(282)
nimodipine	agonist	0.8 ± 1.3		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(204)
nicardipine	agonist	0.5 ± 0.07		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(204)
nitrendipine	agonist	3.8 ± 0.3		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(204)
(±) BayK8644	agonist	32.7 ± 0.2		mTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging	(204)
lidocaine	agonist	5700 ± 200 24000 ± 600		rTRPA1 electrophysiology in HEKT cells (–60 mV) hTRPA1 electrophysiology in HEKT cells (–60 mV)	(393)
5-nitro-2-(3-phenylpropylamino)benzoic acid (NPPB)	agonist	0.32		hTRPA1-expressing HEKT cells using FLIPR	(409)
propofol (2,6-diisopropylphenol)	bimodal	65.4 2.4 17	 19.5	hTRPA1 electrophysiology in HEKT cells (–60 mV) mTRPA1 electrophysiology in CHO cells mTRPA1-expressing Sf21 cells using Flexstation III	(519) (319) (761)

thymol	bimodal	64 127 20 < 100	 > 100	hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging hTRPA1 electrophysiology in HEKT cells (–80 mV) hTRPA1-expressing HEKT cells using FLIPR mTRPA1 electrophysiology in CHO cells	(391) (391) (318)
menthol	bimodal	95 ± 15 278 ± 30 5.2 ± 0.7 7.1 ± 1.1	56 ± 8 68 > 1000 950 ± 80 511 ± 25	mTRPA1 electrophysiology in CHO cells mTRPA1 electrophysiology in CHO cells (-60 mV) hTRPA1 mTRPA1 rTRPA1-expressing HEKT cells using FLIPR	(318) (419) (90)
1-hexanol (1-C6OH)	agonist	7900 ± 900		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(357)
1-heptanol (1-C7OH)	agonist	2700 ± 400		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(357)
1-octanol (1-C8OH)	agonist	810 ± 20		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(357)
apomorphine	agonist	7.1		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(617)
6-(methylsulfinyl)hexyl isothiocyanate (6-MSITC)	agonist	150 ± 30 39 ± 4		mTRPA1 hTRPA1 electrophysiology in HEKT cells (-60 mV)	(710)
6-(methylthio)hexyl isothiocyanate (6-MTITC)	agonist	30 ± 3 34 ± 3		mTRPA1 hTRPA1 electrophysiology in HEKT cells (-60 mV)	(710)
cannabinol (CBN)	agonist	0.18 ± 0.02		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(157)

cannabichromene (CBC)	agonist	0.06 ± 0.02		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(157)
cannabidiol (CBD)	agonist	0.096 ± 0.012 0.11 ± 0.05		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(157) (155)
Δ9-tetrahydrocannabinol acid (THCA)	agonist	0.24 ± 0.03 2.7 ± 0.9		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(157) (155)
cannabidiol acid (CBDA)	agonist	12 ± 9 5.3 ± 1.5		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(157) (155)
cannabigerol (CBG)	agonist	3.4 ± 1.0 0.7 ± 0.03		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(157) (155)
cannabigerol acid (CBGA)	agonist	8 ± 4		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(155)
cannabigivarin (CBGV)	agonist	1.60 ± 0.01		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(155)
tetrahydrocannabivarin (THCV)	agonist	1.5 ± 0.6		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(155)
tetrahydrocannabivarin acid (THCVA)	agonist	16 ± 2		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(155)
anandamide (AEA)	agonist	10 ± 2		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(156)
9-hydroxyoctadecadienoic (9-HODE)	agonist	32 ± 4		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(156)

13-hydroxyoctadecadienoic (13-HODE)	agonist	13 ± 2		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(156)
arachidonic acid (AA)	agonist	13 ± 4		hTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(583)
R-(+)-(2,3-dihydro-5-methyl-3-[(4-morpholinyl)methyl]pyrol [1,2,3-de]-1,4-benzoxazin-6-yl)-(1-naphthalenyl) methanone mesylate (WIN)	agonist	18 20 ± 6		mTRPA1 electrophysiology in CHO cells (-60 mV) rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(6) (641)
(R,S)-3-(2-iodo-5-nitrobenzoyl)-1-(1-methyl-2-piperidinylmethyl)-1H-indole (AM1241)	agonist	48		mTRPA1 electrophysiology in CHO cells (-60 mV)	(6)
N-(2-chloroethyl)-5Z,8Z,11Z,14Z-eicosatetraenamide (ACEA)	agonist	12		mTRPA1 electrophysiology in CHO cells (-60 mV)	(6)
AM251	agonist	0.86 ± 0.06		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(641)
AM630	agonist	1.9 ± 0.2		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(641)
deacylasadisulfide propionate	agonist	11.0 ± 1.4		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(632)
deacylasadisulfide arachidate	agonist	11.0 ± 1.4		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(632)
asadisulfide alcohol	agonist	10.9 ± 0.8		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(632)

foetisulfide A	agonist	11 ± 4		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(632)
isovelleral	agonist	0.50 ± 0.13 2.6 ± 1.1		hTRPA1 mTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(199)
polygodial	agonist	0.40 ± 0.07 0.059 0.67		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging hTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging mTRPA1 electrophysiology in HEKT cells (-60 mV)	(199)
miogatrial	agonist	0.13 0.63		hTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging mTRPA1 electrophysiology in HEKT cells (-60 mV)	(199)
miogadial	agonist	0.2 0.4		hTRPA1-expressing CHO cells using intracellular Ca^{2+} imaging mTRPA1 electrophysiology in HEKT cells (-60 mV)	(199)
crotonaldehyde	agonist	23		rTRPA1- expressing HEKT cells using intracellular Ca^{2+} imaging	(33)
hydroxy-α-sanshool	agonist	69		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(585)
6-shogaol	bimodal	11.2 16 ± 2	 16.7 ± 0.4	hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging rTRPA1-expressing HEKT cells intracellular Ca^{2+} imaging	(585) (477)
6-paradol	agonist	71		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(585)
linalool	agonist	117		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(585)

carvacrol	agonist	750 ± 110 7		WC frog TRPA1 electrophysiology in oocytes (-60 mV) hTRPA1-expressing HEKT cells using FLIPR	(603) (391)
eugenol	agonist	260		hTRPA1 electrophysiology in HEKT cells (-60 mV)	(138)
1'S-1'-acetoxychavicol acetate (ACA)	agonist	0.16		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(497)
2-tert-butyl-5-methylphenol	agonist	3		hTRPA1-expressing HEKT cells using FLIPR	(391)
2,6-dimethylphenol	agonist	31		hTRPA1-expressing HEKT cells using FLIPR	(391)
2,5-dimethylphenol	agonist	57		hTRPA1-expressing HEKT cells using FLIPR	(391)
3,4-dimethylphenol	agonist	67		hTRPA1-expressing HEKT cells using FLIPR	(391)
2,6-diisopropylphenol	agonist	4		hTRPA1-expressing HEKT cells using FLIPR	(391)
caffeine	bimodal	96 ± 11 62 ± 3 1000-2500	 990 ± 120	rTRPA1 mTRPA1 hTRPA1-expressing HEKT cells using FLIPR mTRPA1-expressing HEKT cells intracellular Ca^{2+} imaging	(90) (488)
trinitrophenol (TNP)	agonist	107 ± 6 30 ± 5		hTRPA1 mTRPA1-expressing HEKT cells using FLIPR	(90)

farnesyl thiosalicylic acid (FTS)	agonist	4.9 ± 0.9 86 ± 13 100 ± 10		hTRPA1 mTRPA1 rTRPA1-expressing HEKT cells using FLIPR	(90)
3'-carbamoylbiphenyl-3-yl cyclohexylcarbamate (URB597)	agonist	8 ± 2 74 ± 20 129 ± 23		hTRPA1 mTRPA1 rTRPA1-expressing HEKT cells using FLIPR	(90)
4-methyl-N-[2,2,2-trichloro-1-(4-nitro-phenylsulfa-nyl)-ethyl]-benzamide (CMP1)	bimodal	0.93 ± 0.05 0.88 ± 0.03	 1.0 ± 0.1 2.7 ± 0.3	mTRPA1 rTRPA1-expressing HEKT cells using FLIPR hTRPA1 rhTRPA1-expressing HEKT cells using FLIPR	(90)
6-gingerol	bimodal	10.4 ± 0.03	> 100	rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(477)
L-carveol	agonist	190 ± 30		hTRPA1-expressing Flp-In 293 cells using intracellular Ca^{2+} imaging	(471)
trans-p-methoxycinnamaldehyde	agonist	30 ± 15		hTRPA1-expressing Flp-In 293 cells using intracellular Ca^{2+} imaging	(471)
methyl eugenol	agonist	160 ± 20		hTRPA1-expressing Flp-In 293 cells using intracellular Ca^{2+} imaging	(471)
4-allylanisole	agonist	1500 ± 300		hTRPA1-expressing Flp-In 293 cells using intracellular Ca^{2+} imaging	(471)
p-anisaldehyde	agonist	550 ± 70		hTRPA1-expressing Flp-In 293 cells using intracellular Ca^{2+} imaging	(471)
piperine	agonist	30		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(539)

isopiperine	agonist	33		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(539)
isochavicine	agonist	71		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(539)
piperanine	agonist	150		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(539)
piperolein A	agonist	7.8		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(539)
piperolein B	agonist	11		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(539)
N-isobutyl-(2E,4E)-tetradeca-2,4-diamide (N-tetra)	agonist	19		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(539)
curcumin	agonist	3.3		rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(494)
oleocanthal	agonist	2.8		hTRPA1 electrophysiology in HEKT cells (-60 mV)	(567)
umbellulone	bimodal	19 ± 4 28 ± 7	 410 ± 50	rTRPA1 hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging mTRPA1 electrophysiology in CHO cells	(504) (801)
dihydroumbellulone	bimodal	22	340 ± 80	mTRPA1 electrophysiology in CHO cells	(801)
tetrahydroumbellulone	bimodal	ND	380 ± 30	mTRPA1 electrophysiology in CHO cells	(801)
β-umbellulol	bimodal	ND	420 ± 40	mTRPA1 electrophysiology in CHO cells	(801)

acetyl tetrahydroumbellulone	bimodal	ND	490 ± 60	mTRPA1 electrophysiology in CHO cells	(801)
acetyl β-umbellulol	bimodal	ND	> 1000	mTRPA1 electrophysiology in CHO cells	(801)
ligustilide	bimodal	44	1500	mTRPA1 electrophysiology in CHO cells	(801)
dehydroligustilide	bimodal	540	23	mTRPA1 electrophysiology in CHO cells	(801)
capsiate	agonist	2.76 ± 0.08		hTRPA1 electrophysiology in HEKT cells (-60 mV)	(631)
dihydrocapsiate	agonist	2.9 ± 0.2		hTRPA1 electrophysiology in HEKT cells (-60 mV)	(631)
nordihydrocapsiate	agonist	2.82 ± 0.16		hTRPA1 electrophysiology in HEKT cells (-60 mV)	(631)
artepillin C	agonist	1.8		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(254)
baccharin	agonist	16		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(254)
drupanin	agonist	> 250		hTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(254)
methyl syringate	agonist	510		hTRPA1-expressing Flp-In 293 cells intracellular Ca^{2+} imaging	(647)
perillaldehyde	agonist	41 ± 8 42 ± 8		rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(65) (64)

perillaketone	agonist	22 ± 2 20 ± 2		rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging rTRPA1-expressing HEKT cells using intracellular Ca^{2+} imaging	(65) (64)

Table 4. TRPA1 inhibitors

Name	Company	IC_{50}	References	Comments
HC-030031	Hydra Biosciences, Inc.: USA	$\leq$ 1.8-20 μM	WO2007073505 (2007); (194, 422, 444)	Non-electrophilic. Effective in human, rat, mouse, guinea pig. Ineffective on frog and zebrafish channels (7)
Hydra 7	Hydra Biosciences, Inc.: USA	$\leq$ 10 μM	WO2009002933 (2008)	
Chembridge-5861528	Hydra Biosciences, Inc.: USA	14-18 μM	(750)	
CB-625	Cubist Pharmaceuticals/Hydra Biosciences	N.D.		Phase I clinical trial completed. Discontinued due to solubility concerns (17)
Glenmark 10	Glenmark Pharmaceuticals, SA (Switzerland)	50-100 nM	US2009325987 (2009)	
Glenmark 15	Glenmark Pharmaceuticals, SA (Switzerland)	< 50 nM	US2009325987 (2009)	
Glenmark 37	Glenmark Pharmaceuticals, SA (Switzerland)	< 50 nM	US2009325987 (2009)	
Glenmark 17	Glenmark Pharmaceuticals, SA (Switzerland)	< 250 nM	WO2009118596 (2009)	
Glenmark 23	Glenmark Pharmaceuticals, SA (Switzerland)	0.5- 1 μM	WO2009118596 (2009)	
Glenmark 8	Glenmark Pharmaceuticals, SA (Switzerland)	< 500 nM	WO2009144548 (2009)	
Glenmark 39	Glenmark Pharmaceuticals, SA (Switzerland)	< 500 nM	WO2009144548 (2009)	
GRC-17536	Glenmark Pharmaceuticals, SA (Switzerland)	< 10 nM	(489)	Phase IIa clinical trial (NCT01726413)

				Diabetic peripheral neuropathy/Respiratory disorders
N,N´-bis-(2-hydroxybenzyl)-2,5- diamino-2,5-dimethylhexane	IRM LLC, A Delaware Limited Liability Company, Bermuda	N.D.	WO2007098252 (2007)	
tramadol	Grünenthal GmbH	0.1-10 μM	(466)	Formerly known as Tramal. First launched and marketed by Grünenthal GmbH in 1977.
AMD_09	University of Florence, Italy	10.3-13.2 μM	(244)	
AMD_12	University of Florence, Italy	7.3-8.2 μM	(244)	
AP-18	IRM LLC, A Delaware Limited Liability Company, Bermuda	3.1 μM	(570) WO2007098252 (2007)	
A-967079	Abbott Laboratories	67-290 nM	WO2009089082 (2009) (128)	
Renovis 11	Renovis, Inc. (a wholly-owned subsidiary of Evotec AG)	2.7 μM	(163)	
AZ456	AstraZeneca	30-305 nM	WO2012050512 (2012) (530, 720)	
AMG7160	Amgen Inc.	51 nM	(346)	
AMG2504	Amgen Inc.	35 nM	(346)	
AMG9090	Amgen Inc.	21 nM	(346)	
AMG5445	Amgen Inc.	91 nM	(346)	
CMP1	Abbott Laboratories	2 μM	(131)	
CMP2	Abbott Laboratories	1.4 μM	(131)	
CMP3	Abbott Laboratories	1.1 μM	(131)	
2B10	Amgen Inc.	90-260 nM	(395)	(monoclonal antibody)

SZV-1287	University of Pécs, Hungary	2.4 μM	(566)	
JNJ-41477670	Janssen Pharmaceuticals	7.2 nM	(89)