Reviews of Physiology, Biochemistry and Pharmacology 167

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Contents

The TRPA1 Channel in Inflammatory and Neuropathic Pain and Migraine

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Abstract The transient receptor potential ankyrin 1 (TRPA1), a member of the TRP superfamily of channels, is primarily localized to a subpopulation of primary sensory neurons of the trigeminal, vagal, and dorsal root ganglia. This subset of nociceptors produces and releases the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP), which mediate neurogenic inflammatory responses. TRPA1 is activated by a number of exogenous compounds, including molecules of botanical origin, environmental irritants, and medicines. However, the most prominent feature of TRPA1 resides in its unique sensitivity for large series of reactive byproducts of oxidative and nitrative stress. Here, the role of TRPA1 in models of different types of pain, including inflammatory and neuropathic pain and migraine, is summarized. Specific attention is paid to TRPA1 as the main contributing mechanism to the transition of mechanical and cold hypersensitivity from an acute to a chronic condition and as the primary transducing pathway by which oxidative/nitrative stress produces acute nociception, allodynia, and hyperalgesia. A series of migraine triggers or medicines have been reported to modulate TRPA1 activity and the ensuing CGRP release. Thus, TRPA1 antagonists may be beneficial in the treatment of inflammatory and neuropathic pain and migraine.

Keywords Migraine · Nociceptors · Pain · TRPA1

Contents

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1   TRP Channels and Sensory Neurons

The original discovery that vision in *Drosophila* is produced by an initial activation of a transient inward current associated with receptor stimulation (Montell and Rubin 1989) has led, with an unprecedented pace, to the identification of the transient receptor potential (TRP) channels. TRPs represent one of the largest families of ion channels with more than 56 subtypes, which are widely distributed within the phylogeny where they contribute to an array of different physiological functions and are implicated in a series of pathological conditions. In mammals, TRPs comprise 28 membrane proteins mainly behaving as non-selective cation permeable channels. TRPs are classified into seven subfamilies: TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPP ('Polycystin'), TRPML ('Mucolipin'), TRPA ('Ankyrin'), and TRPN ('NOMP-C') (see also (Nilius 2007)).

1.1   TRP Channels: Structure and Mechanism of Activation

TRP's general structure recapitulates that of voltage-gated channels with six transmembrane domains (S1-6) and the intracellular N- and C-terminal regions of variable length with a pore loop between S5 and S6 (Owsianik et al. 2006; Nilius 2007). It has been reported that four subunits, each composed of six

transmembrane domains, may assemble in homo- and/or hetero-tetramers to form the functional channel, where each subunit contributes to a shared selectivity filter and ion-conducting pore (Schaefer 2005). Beyond their general membrane topology and permeability to cations, TRP channels are strikingly diverse and exhibit a wide variety of modes of activation by chemical and physical stimuli (exogenous chemical compounds, lipids, oxidative stress, acids, pheromones, osmolarity, mechanical stimulation, light, temperature, and others), regulatory mechanisms (transcription, alternative splicing, glycosylation, phosphorylation), and tissue distribution (virtually all cells tested express at least one member of the super-family). These features underline the unprecedented diversity of physiological and pathophysiological functions mediated by TRPs and their definition as polymodal sensors.

1.2 TRP Channels and Nociceptors

TRP channels are expressed in cellular membranes, with the exception of the nuclear envelope and mitochondria, of almost every excitable and non-excitable cell type. The object of the present review is finalized to describe physiological and pathophysiological functions of the TRP channels localized to a subset of primary sensory neurons (Szallasi et al. 2007), where they result highly involved in sensing physiological and noxious agents and, more generally, in pain perception. Just about a century ago, Sherrington proposed the existence of nociceptors, a subgroup of primary sensory neurons which are activated by tissue damaging stimuli, such as heat, intense pressure, or irritant chemicals, but not by innocuous stimuli such as warming or light touch (Sherrington 1906; Julius and Basbaum 2001). The heterogeneous population of primary sensory neurons and the fibres that they originate can be differentiated according to morphological, electrophysiological, neurochemical, functional, and other criteria. In particular, neurons with the largest diameter cell body give rise to myelinated, rapidly conducting Aβ fibres, which detect innocuous stimuli and normally do not contribute to nociceptive stimulus transduction. In contrast, neurons with small- and medium-diameter cell bodies give rise to un-myelinated, slowly conducting C-fibres and thinly myelinated, more rapidly conducting Aδ-fibres. Both are highly involved in nociception. Aδ- and C-nociceptive fibres either respond to a single type of physical stimuli, or more commonly integrate and generate responses to potentially damaging thermal, mechanical, and/or chemical stimuli, and for this reason are also defined as polymodal nociceptors (Julius and Basbaum 2001).

A specific subset of C-fibre and Aδ-fibre nociceptors is exquisitely sensitive to capsaicin, the pungent ingredient in hot peppers, and for this reason they have been labelled as 'capsaicin-sensitive' sensory neurons. The selective excitatory role of capsaicin is associated in a time- and concentration/dose-dependent manner with the ability of the compound to desensitize the channel and to defunctionalize TRPV1-positive nociceptors to capsaicin itself and to any other stimulus. This

unique property of capsaicin most likely derives from excessive Ca^{2+} influx through the channel, which, in adult rats transiently and in newborn rats permanently, alters nociceptor morphology and functioning (Bevan and Szolcsanyi 1990; Szallasi and Blumberg 1999; Szallasi et al. 2007; O'Neill et al. 2012). This pharmacological property of capsaicin to produce non-specific nociceptor defunctionalization has been exploited therapeutically for topical treatment of patients affected by neuropathic pain of viral origin (Backonja et al. 2008).

TRPV1-expressing neurons comprise a subcategory defined as peptidergic because they produce the neuropeptides calcitonin gene-related peptide (CGRP) and the tachykinins, substance P (SP) and neurokinin A (NKA), which in response to depolarization, capsaicin or other excitatory stimuli, are released from central and peripheral neuronal terminals. Upon peripheral neuropeptide release, activation of CGRP and tachykinin (NK_1, NK_2 and NK_3) receptors on effector cells, particularly at the vascular level, causes a series of inflammatory responses, collectively referred to as "neurogenic inflammation" (Geppetti and Holzer 1996). Given their relevance in migraine and other diseases, neurogenic inflammatory responses will be further discussed in Sects. 5 and 6. The peculiar property of capsaicin, first to excite and thereafter to desensitize both afferent (nociception) and efferent (neurogenic inflammation) responses, has given an unprecedented contribution to our current understanding of the role of these neurons in health and disease. In addition to TRPV1, peptidergic nociceptors also express other TRPs, including the TRPV2, TRPV3, and TRPV4 channels and TRPA1 (Story et al. 2003), whereas TRPM8 seems to be confined to non-peptidergic sensory neurons (Bhattacharya et al. 2008). Because of the property to sense temperatures from cold (A1 and M8) to warm (V3 and V4) and hot (V1 and V2), these channels have been collectively labelled as thermoTRPs (Guler et al. 2002; Watanabe et al. 2002; Story et al. 2003). Exogenous agents, recognized early on as thermoTRP stimulants, include camphor for TRPV3 and TRPV4 (Moqrich et al. 2005), menthol for TRPM8 (McKemy et al. 2002; Peier et al. 2002), and mustard and cinnamon oil for TRPA1 (Bandell et al. 2004; Jordt et al. 2004). The ability to sense, in addition to variations in temperature, physical and chemical changes within nerve terminal milieu indicates thermoTRPs as molecular integrators of multiple sensory modalities. Finally, coexistence of neuropeptides and TRPs in the same sensory nerve terminals implies that different channel (TRPV1, TRPV4, or TRPA1) activation may drive the release mechanism that eventually results in the protective and/or detrimental process promoted by neurogenic inflammation (Geppetti and Holzer 1996).

2   TRPA1 Channel

From its first cloning (Jaquemar et al. 1999), the ankyrin-1 subtype of the TRP superfamily has gained increasing scientific interest because of its role as a sensor of irritating and cell-damaging agents. In particular, the identification of TRPA1 as the target of an unprecedented series of chemically diverse molecules, many of

which are generated following oxidative stress, points to the TRPA1-oxidative stress system as a novel pathway to produce pain and neurogenic inflammation. Although previous reports have identified the main role of TRPA1 in nociceptive pain models, more recent studies have emphasized the key function of the channel in models of neuropathic pain (particularly in the transition from acute nociception to chronic hypersensitivity) and in models of those peculiar types of pain experienced by migraine or cluster headache patients.

2.1 Structure and Functions

The ankyrin-like protein with transmembrane domains (ANKTD), initially identified in lung fibroblasts (Jaquemar et al. 1999), has been successively reclaimed as a TRP member for its strong homology with several members of the superfamily (Story et al. 2003). In humans, the *trpa1* gene consists of 27 exons and spans 55,701 base pairs of the human chromosome 8q13. TRPA1, found in mammals and invertebrates, including mouse, rat, dog, chicken, zebrafish, fruit fly, and *Caenorhabditis elegans* (Nilius et al. 2012), is a protein of about 1,100 aminoacids (120–130 kDa), but shorter splice variants have also been identified. Like all TRP channels, TRPA1 has six transmembrane domains (S1-6) with a pore region between S5 and S6 and cytoplasmic N- and C- terminal regions. The peculiarity of TRPA1 is a particularly elongated (14–18) ankyrin repeat region within the N-terminus, which can connect transmembrane proteins to the cytoskeleton and can be involved in protein–protein interactions, as well as in channel trafficking to the plasma membrane. In its functional configuration, TRPA1 forms tetramers, usually homo-tetramers. TRPA1 subunits may often co-localize with TRPV1 channels, thereby assembling into hetero-tetrameric complexes to adapt the single channel biophysical properties in native sensory neurons (Staruschenko et al. 2010; Nilius and Owsianik 2011). In particular, direct interaction between TRPA1 and TRPV1 and regulation of TRPA1 intrinsic characteristics by the TRPV1 appear to derive from a complex made up by TRPA1 and TRPV1 proteins (Staruschenko et al. 2010). However, the intriguing hypothesis that functional interaction is dependent on, not intracellular Ca^{2+} but rather direct interaction (Staruschenko et al. 2010) between the two channels, has not received conclusive proof.

TRPA1, like the majority of TRPs, behaves as a non-selective cation channel with a typically inward depolarizing current prevalently due to Na^+ and Ca^{2+} ions (Nilius et al. 2007). The constitutive open TRPA1 channel evokes outwardly rectifying currents, which rapidly inactivate at positive potentials, although the nature of this inactivation is still unknown. TRPA1 activation by electrophilic compounds results in large inward currents whereas the outward rectification is mostly abolished (Nilius et al. 2007). In contrast, TRPA1 activation with non-electrophilic compounds still displays outward rectification. The reason for these differences remains to be elucidated. It has been suggested that the extracellular calcium level affects TRPA1 currents. In fact, in the presence of extracellular

Ca^{2+}, activated TRPA1 currents decline rapidly (decay, desensitization), whereas in its absence, both current activation and decay are delayed (Wang et al. 2008).

The N-terminal cysteine residues represent another important functional site of the channel. Indeed, this region, enriched with several key cysteine residues, through the possible formation of a disulfide bonding among them, is required for activation operated by electrophilic molecules (Macpherson et al. 2007; Andersson et al. 2008; Wang et al. 2008), as well as for channel desensitization (Ibarra and Blair 2013). TRPA1 possesses additional domains that appear to be crucial for its function. On the N-terminal region, a putative EF-hand motif has been identified and represents the most common mechanism for a large number of Ca^{2+}-interacting proteins. It is known that intracellular Ca^{2+} ions potentiate agonist-induced responses and directly activate the channel, probably through this mechanism (Doerner et al. 2007; Zurborg et al. 2007), although its functional relevance is still under debate. TRPA1 channel activity undergoes modulation by negatively charged ligands, including phosphoinositides or inorganic polyphosphates (Samad et al. 2011), interacting with a yet unidentified positively charged domain in the C-terminal region. Basic residues in the C-terminus, strongly involved in TRPA1 voltage and chemical sensitivity, may represent the possible interaction sites for negatively charged molecules that are generally considered to modulate TRPA1 (Samad et al. 2011).

2.2   Neuronal and Extra-Neuronal Localization

As already mentioned, TRPA1 is abundantly expressed by a subpopulation of primary sensory neurons of the dorsal root (DRG), trigeminal (TG), and vagal (VG) ganglia. TRPA1-expressing neurons have unmyelinated C- and thinly mye-linated Aδ-fibres, and only occasionally large myelinated fibres (Story et al. 2003; Bhattacharya et al. 2008). However, more recent evidence of co-localization of TRPA1 with markers of non-peptidergic neurons, including the purinergic P2X3 receptor, isolectin B4 (IB4), or the Na(V)1.8 channel (Kim et al. 2010, 2011; Barabas et al. 2012), has challenged the previous proposal that TRPA1 expression is completely confined to peptidergic nociceptors (Story et al. 2003; Bhattacharya et al. 2008). Additional localization of TRPA1 in the CNS may have some functional relevance. These include the hippocampal neurons, where it seems to be linked with the cannabinoid receptor CB1 (Koch et al. 2010) and the astrocytes, where it appears to contribute to resting intracellular Ca^{2+} levels and regulating inhibitory synapses modulating the extracellular concentration of γ-aminobutirric acid (Shigetomi et al. 2011; Lee et al. 2012) or to be required for constitutive d-serine release into the extracellular space, thus contributing to NMDA receptor-dependent long-term potentiation (Shigetomi et al. 2013).

In the last 5 years emerging evidence has identified TRPA1 in a variety of extra-neuronal tissues, where it contributes to different regulatory and proinflammatory pathways. TRPA1 is expressed in: the inner ear and the organ of

Corti, where it appears to play a role in mechanical transduction (Garcia-Anoveros and Duggan 2007); vascular endothelial cells (Earley et al. 2009), where it modulates vessel tone; keratinocytes and skin fibroblasts, where it mediates secretion of eicosanoids, such as prostaglandin E_2 (PGE_2) and leukotriene B_4 (LTB_4), thereby promoting erythema (Jain et al. 2011); rat pancreatic islets, where it facilitates insulin release (Cao et al. 2012); several cell types of the gastrointestinal tract, where it senses the chemical environment and modulates gastrointestinal motility (Nozawa et al. 2009; Poole et al. 2011; Kono et al. 2013); dental pulp fibroblasts, where it contributes to the perception of noxious cold and to cold hypersensitivity (Kim et al. 2012); airway and lung fibroblasts and epithelial and smooth muscle cells, where it modulates interleukin-8 release (Mukhopadhyay et al. 2011; Nassini et al. 2012b). This novel scenario, broader than previously predicted, shed a new light on the more heterogeneous and complex distribution of TRPA1, and its possible participation in physiological functions and pathological conditions beyond the nervous system (Kang et al. 2010).

2.3 TRPA1 Agonists

TRPA1 agonists, intended as both direct activators and modulators, encompass an exceedingly high number of heterogeneous molecules, considering their source or chemical structure. According to the mechanism of activation, TRPA1 agonists can be divided into two main groups: electrophilic or non-electrophilic. The majority belong to the first group which gates the channel by modifying or interacting with nucleophilic cysteine and lysine residues of the channel N-terminus (Hinman et al. 2006; Macpherson et al. 2007). Covalent modification of the aminoacidic residues can occur in several chemical ways, including Michael addition, formation of a thiocarbamate intermediate, and generation of cysteine–disulfide products or alkylation (Cebi and Koert 2007). In addition to the huge number of electrophilic activators, TRPA1 can also be activated through non-covalent protein modifications. Non-electrophilic compounds often behave via bimodal mechanism. Low concentrations activate, whereas higher concentrations inhibit, channel activity, as in the case of menthol, apomorphine (Schulze et al. 2013), or nicotine (Karashima et al. 2007; Kichko et al. 2013).

According to their source, TRPA1 agonists can be classified into two main groups. The first group encompasses exogenous compounds, which include molecules derived from natural or alimentary origin, drugs or drug metabolites, and, last but not least, environmental irritant molecules. The second group is represented by a growing series of endogenously produced compounds, mainly generated under inflammatory conditions or after tissue injury. There are several cases where the distinction between exogenous and endogenous appears to be ambiguous, as in the case of acrolein, an α,β-unsaturated aldehyde and highly reactive molecule which is generated by combustion in vehicle exhaust, was used as a tear gas, is a metabolite of the chemotherapeutic agent cyclophosphamide, but is also endogenously

produced by oxidative stress via peroxidation of plasma membrane phospholipids. Nevertheless, this latter subdivision will be adopted for the description of individual agonists because of its key relevance in relation to putative pathophysiological roles of TRPA1.

2.4 Exogenous Agonists

Exogenous agonists encompass a large variety of compounds in terms of source, chemical structure and possible pathophysiological role. In particular, according to current knowledge, several TRPA1 agonists derive from the vegetal realm, where evolutionary pressure could have promoted the development of ingenious defensive systems to ward off herbivorous predators. Many natural compounds, with irritant properties, have been useful to better characterize the role of this channel in many pathophysiological conditions, such as pain or inflammation. Among this abundant group of compounds, the activators best known either for their potency and/or for their selectivity are cinnamaldehyde, contained in the cinnamon oil extracted from the *Cinnamomum* (Bandell et al. 2004), several isothiocyanate compounds, such as allyl or benzyl isothiocyanate contained in mustard oil or wasabi, obtained from the *Brassica* seeds (Jordt et al. 2004), and allicin and diallyl disulfide, contained in garlic (*Allium sativum*) (Bautista et al. 2005). These compounds share a common reactive chemical structure, which enables them to covalently modify specific cysteine residues, located within the cytoplasmic N-terminal region of the channel (Hinman et al. 2006), resulting in TRPA1 activation and the consequent nociceptive response. These features justify the widespread use of these compounds to understand the mechanism of action and the role of the channel. Additional less potent or selective molecules have been reported to activate TRPA1. These include gingerol (Bandell et al. 2004), contained in ginger, which also gates TRPV1 (Morera et al. 2012), thymol, a major component of thyme (*Thymus vulgari*) and oregano (*Origanum vulgare*), and carvacrol, contained in oregano (Xu et al. 2006; Lee et al. 2008b) (for a systematic review see Nilius and Appendino 2013).

Elevated concentrations of the non-electrophilic compound, delta-9-tetrahydro-cannabinol (THC), contained in *Cannabis sativa* activate the TRPA1 channel without producing any covalent modification (De Petrocellis et al. 2008). Other phytocannabinoids have been shown to gate the TRPA1 channel (De Petrocellis et al. 2008). Certain medicines or their metabolites represent an additional subgroup of exogenous TRPA1 activators. Indeed, some hitherto unexplained inflammatory or painful adverse reactions might be better understood by the recently identified ability of such drugs/metabolites to stimulate TRPA1. General anaesthetics, including isoflurane, desflurane, sevuflurane, and propofol, are now known to produce neurogenic inflammation and a pungent sensation by exciting Aδ and C fibres in the respiratory tract, via direct activation of TRPA1 (Matta et al. 2008; Eilers et al. 2009; Satoh and Yamakage 2009; Patwardhan et al. 2012). Membrane permeable local anaesthetics, including lidocaine (at high concentrations), gate

TRPA1 by both covalent and irreversible modification of intracellular cysteine residues and by interacting with the S5 transmembrane domain (Leffler et al. 2011). Topical application of nicotine for replacement therapies causes irritation of the mucosa and skin, an effect long attributed to the activation of nicotinic acetylcholine receptors in chemosensory neurons (Dussor et al. 2003). However, it has recently been demonstrated that nicotine is able to activate TRPA1 in nociceptors, thereby exerting a fundamental role in the associated irritant response (Talavera et al. 2009). In particular, nicotine seems to act in a membrane-delimited manner, stabilizing the open state and destabilizing the closed state of the channel (Talavera et al. 2009).

Prostaglandins are known to sensitize nociceptors, and non-steroidal antiinflammatory drugs (NSAIDs) or selective cyclooxygenase II inhibitors (COXibs) exert their analgesic activity by inhibiting prostaglandin synthesis (Mitchell and Warner 1999). The emerging role of TRPA1 in inflammatory pain has focused attention on the possibility that NSAIDs sensitize the channel. Paradoxically, fenamate NSAIDs, including flufenamic, niflumic, and mefenamic acid, have been shown to activate the TRPA1 channel in several in vitro models (Hu et al. 2009) and several arylalkanoic acids, such as diclofenac and indomethacin, and 2-arylpropionic acids, such as flurbiprofen and ketoprofen, have been shown to activate in vitro rodent and human TRPA1 (Hu et al. 2009). However, there are no reports about the in vivo pro-algesic or proinflammatory effect of these drugs via TRPA1, thus confining these findings to a rather limited clinical impact. An additional and heterogeneous series of currently used drugs, including the antimycotic clotrimazole (Meseguer et al. 2008), the antidiabetic glibenclamide (Babes et al. 2013), the non-narcotic morphine derivative, apomorphine (Schulze et al. 2013), the antirheumatic medicine, auranophin (Hatano et al. 2013), the antihypertensive and antianginal dihydropyridines (Fajardo et al. 2008), have been shown to activate TRPA1. Again, the low potency of all these drugs to target the channel suggests that, if any, the clinical significance of such findings is poor. Apart from direct activators, there is a rising group of drugs, which indirectly activates and/or sensitizes the channel. This novel mechanism will be discussed in more detail below.

Finally, a rather broad group of environmental irritants has been qualified as TRPA1 channel stimulants. These, as reported above, include acrolein (Bautista et al. 2006). Tear gases (CN, CR and CS) are TRPA1 activators (Brone et al. 2008). Additional aldehydes, which stimulate TRPA1, are formaldehyde (McNamara et al. 2007), acetaldehyde (Bang et al. 2007) and crotonaldehyde (Andre et al. 2008, 2009) (all contained in cigarette smoke). The intriguing hypothesis that toxic inhalation hazards produce conditions labelled as work-related asthma or airways dysfunction syndrome (RADS) (Brooks and Bernstein 2011; Geppetti et al. 2014), or chronic obstructive pulmonary disease (COPD), via TRPA1 activation, is substantiated by recent observations. Indeed, toluene diisocyanate, which is considered the causative agent of occupational asthma (Ott et al. 2003), gates TRPA1 (Taylor-Clark et al. 2008a) to trigger airway inflammation.