



Transient receptor potential channels and occupational exposure

Pierangelo Geppetti^a, Riccardo Patacchini^{a,b}, and Romina Nassini^a

Purpose of review

The discovery that a number of transient receptor potential (TRP) channels are expressed in a subpopulation of primary sensory neurons innervating the upper and lower airways as well as in nonneuronal cells in the airways and lungs has initiated a quest for the understanding of their role in the physiology and pathophysiology of the respiratory tract.

Recent findings

Various members of the TRP vanilloid subfamily (TRPV1, TRPV4) and the TRP ankyrin 1 (TRPA1), because of their localization in peptidergic sensory neurons, promote airway neurogenic inflammation. In particular, TRPA1, which is gated by oxidative and nitrate stress byproducts, has been found to mediate inflammatory responses produced by an unprecedented series of toxic and irritant agents produced by air pollution, contained in cigarette smoke, and produced by accidental events at the workplace. The observation that reactive molecules endogenously produced in the airways/lungs of asthma, work-related asthma, and chronic obstructive pulmonary disease target TRPA1 underscores the primary role of the TRPA1 channel in these conditions.

Summary

Identification of TRP channels, and especially TRPA1, as major targets of oxidative/nitrate stress and a variety of irritant environmental agents supports the hypothesis that neurogenic inflammation plays an important role in work-related inflammatory diseases and that antagonists for such channels may be novel therapeutic options for the treatment of these diseases.

Keywords

neurogenic inflammation, nitrate stress, oxidative stress, TRPA1, TRPV1

INTRODUCTION

The environment is an unlimited source of agents that directly or indirectly interact with the human body at the level of exposed surfaces, primarily represented by the skin, the gastrointestinal tract and, because of the continuous and vital influx of inhaled air, the respiratory system. These agents in the vast majority of cases exert an inert action, but in tobacco smoking or under specific circumstances, such as in the workplace, accidents, catastrophes, and other similar conditions, potentially harmful substances enter the airways and lungs. Therefore, it is not surprising that the upper and lower airways possess systems specifically suited to immediately sense and recognize harmful agents. A subset of primary sensory neurons are distinguished by their ability to sense dangerous stimuli and to react to them by releasing proinflammatory neuropeptides, which orchestrate an immediate protective response, labelled as 'neurogenic inflammation'. However, as for other inflammatory pathways, also

neurogenic inflammatory responses, under chronic circumstances, may contribute to the mechanism of diseases, including asthma, chronic obstructive pulmonary disease (COPD), or chronic cough. The discovery that peptidergic primary sensory neurons express some members of the transient receptor potential (TRP) family of channels, which feature the unique property of transducers of physical (mechanical and thermal) and chemical (an unprecedented series of exogenous or endogenous) stimuli [1], has promoted basic and translational research focused on understanding the role of these

^aPharmacology and Oncology Unit, Department of Health Sciences, University of Florence, Florence and ^bChiesi Farmaceutici, Parma, Italy

Correspondence to Pierangelo Geppetti, MD, Department of Health Sciences, University of Florence, Viale Pieraccini, 6, 50139 Florence, Italy. Tel: +39 055 4271329; fax: +39 055 4271280; e-mail: geppetti@unifi.it

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KEY POINTS

- TRPV1 and TRPA1 are expressed by a subpopulation of primary sensory neurons that convey nociceptive input and promote neurogenic inflammation.
- TRPA1 on sensory neurons is a sensor of oxidative and nitrative stress.
- An array of toxic inhalation hazards that cause work-related asthma or contribute to COPD have been recognized to target TRPA1.

channels in protecting the airways and lungs from injury, or contributing to the mechanism of chronic inflammatory diseases.

TRANSIENT RECEPTOR POTENTIAL CHANNELS

The superfamily of the TRP channels, originally discovered in the *Drosophila* as phototransduction proteins, consists of more than 56 subtypes, widely distributed within the phylogeny [2]. The 28 channels identified in mammals are subdivided into seven subfamilies: TRPC ('Canonical'), TRPV ('Vanilloid'), TRPM ('Melastatin'), TRPP ('Polycystin'), TRPML ('Mucolipin'), TRPA ('Ankyrin'), and TRPN ('NOMP-C') (see also [3,4]). TRP channels exhibit a stereotyped structure with S6 transmembrane domains [3,5]; assembly in tetramers and their interactions with ligands are governed by different mechanisms including the channel ankyrin repeats [6]. The low sequence homology of mammalian TRP channels probably is the underlying reason for the ability of the different channels, broadly distributed in practically all tissues and cells, to sense an array of diverse stimuli (temperature, chemical compounds, osmolarity, mechanical stimulation, lipids, light, oxidative stress, acid, pheromones), and to regulate a number of molecular functions (transcription, alternative splicing, glycosylation, phosphorylation).

Stimulation of tyrosine kinase receptors and G protein-coupled receptors via phospholipases C and Ca^{2+} release [7], or direct channel targeting by specific ligands or physical stimuli (thermal or mechanical), result in TRP activation [8]. Regulation of the activity of TRP channels is controlled by posttranslational modification (i.e. phosphorylation) or by protein kinases A, C, and G activity [9]. Because of their ability to transduce and amplify a variety of external or internal signalling molecules, TRPs have been broadly defined as cellular sensors and signal integrators [3].

TRANSIENT RECEPTOR POTENTIAL CHANNELS IN THE AIRWAY SENSORY NEURONS

At least six TRP channels are expressed in a subset of primary sensory neurons. These are the vanilloid 1 (TRPV1), 2 (TRPV2), 3 (TRPV3) and 4 (TRPV4), the TRPM8, and the TRPA1 channels [10,11]. These channels have been collectively labelled as thermo-TRPs because of their property to sense temperatures from cold (A1 and M8) to warm (V3 and V4) and hot (V1 and V2) [10,12,13]. With the exception of TRPM8, all the channels are expressed by peptidergic neurons [10,14]. TRPV1 and TRPA1 may also be expressed by nonpeptidergic neurons [14,15]. The selective ability of capsaicin, the pungent ingredient of hot peppers, to gate TRPV1, producing after an initial stimulation a prolonged or permanent refractoriness to capsaicin itself, and to any other neuronal excitatory stimulus (an effect commonly referred to as 'capsaicin desensitization'), has led to the definition of this subset of nociceptors as 'capsaicin-sensitive' [11]. Neuropeptides contained in and released from capsaicin-sensitive nociceptors include the calcitonin gene-related peptide (CGRP) and the tachykinins substance P and neurokinin A. Their release from central nerve terminals has been associated, with some level of uncertainty, to pain transmission, whereas there is no doubt that tachykinins and CGRP liberated from peripheral nerve endings produce a series of inflammatory responses referred to as 'neurogenic inflammation' [16]. There is now strong clinical evidence suggesting that CGRP-mediated vasodilatation plays a major role in migraine mechanism [17]. It should be underlined that, despite the remarkable number of positive data accumulated in rodents, a similar role for neurogenic inflammation in human asthma and COPD has not yet been established.

TRPV1 AND TRPA1 CHANNELS

The cloning of TRPV1 was a landmark in the study of pain and neurogenic inflammation [18], because it revealed the molecular target of capsaicin that via TRPV1 selective activation, causes its typical pungent sensation and produces all the classical responses collectively referred to as 'neurogenic inflammation'. TRPV1 may also be activated by heat (42–53°C) and a series of endogenous stimuli, including low extracellular pH, anandamide, and certain lipid derivatives [19,20]. The interest in TRPV1 has, however, declined, mainly for two reasons. First, there is much uncertainty regarding the real pathophysiological role in pain and hyperalgesia or other functions of the putative

endogenous agonist(s) that activate TRPV1. Second, and more important, a large series TRPV1 antagonists have been shown to increase body temperature and heat pain threshold in healthy individuals, thus spoiling the clinical development of these compounds [21,22]. Accordingly, the idea of treating cough or other respiratory symptoms with TRPV1 antagonists has been, at least momentarily, hampered.

In contrast, the interest in TRPA1 has been greatly increased by the discovery of the unique ability of an unprecedented series of reactive byproducts of oxidative and nitrative stress to gate the channel. TRPA1, an approximately 1100 amino acid protein, like the majority of TRP channels, is a Ca^{2+} -permeable nonselective cation channel [23]. TRPA1 subunits may co-localize with TRPV1 channels, thus forming heterotetrameric complexes to adapt the single channel biophysical properties [24,25]. Cysteine residues of the N-terminal region are important functional activation sites for electrophilic molecules [26–28]. Reactive molecules undergo nucleophilic attack by specific cysteine residues of TRPA1 by a covalent, but reversible, mechanism known as Michael Addition [26,29], which also modulates channel desensitization [30].

Several common and highly appreciated spices, including cinnamaldehyde, contained in *Cinnamomum* [31], allyl isothiocyanate, contained in mustard oil or wasabi, [32], and allicin and diallyl disulfide, contained in garlic (*Allium sativum*) [33], are strong TRPA1 agonists, while less potent or selective stimulants are gingerol, contained in ginger (which also gate TRPV1) [34], thymol, contained in thyme (*Thymus vulgaris*), and oregano (*Origanum vulgare*) and carvacrol, contained in oregano [35,36] (for a systematic review see [37]). A large series of medicines have been reported to activate TRPA1, although channel activation is only achieved with concentrations exceedingly higher than those achieved in plasma with appropriate dosing [37].

TRPA1 AND OXIDATIVE AND NITRATIVE STRESS

Reactive oxygen species (ROS), including oxygen (O_2) and hydroxyl ($\bullet\text{OH}$) radicals and the nonradical derivatives, superoxide anion ($\text{O}_2^{\bullet-}$) and hydrogen peroxide (H_2O_2), are generated by cellular respiration through the mitochondrial electron transport chain. Nitric oxide and peroxynitrite (ONOO^-) are reactive nitrogen species (RNS) produced under both physiological and pathophysiological circumstances by the activity of nitric oxide synthases. Ionizing radiation, cytotoxic drugs, and infectious

agents are known to increase ROS and RNS. These same reactive molecules are produced under a series of proinflammatory circumstances at the sites of inflammation and during tissue injury. ROS and RNS may react with plasma membrane unsaturated fatty acids to produce highly reactive electrophilic compounds, such as 4-hydroxynonenal (4-HNE), 4-hydroxyhexenal (4-HHE), 4-oxononenal (4-ONE), acrolein, and nitrooleic acid (9-OA- NO_2). Nonenzymatic dehydration of classical prostaglandins (PGs) generates cyclopentenone prostaglandins and isoprostanes, including 15-deoxy- $\Delta^{12,14}$ -PGJ₂ (15-d-PGJ₂), PGA₂ and PGA₁, and 8-isoprostane-PGA₂.

Apart from a few exceptions, for a long time all these molecules have been labelled as by-products of oxidative or nitrative stress that should be quenched, eliminated, or at the best considered as markers of tissue injury. However, recent molecular findings have identified these same molecules as novel mediators to signal pain and neurogenic inflammation. Indeed, a major breakthrough in the last 10 years has been the identification of TRPA1 as the molecular target of ROS, RNS, and other reactive electrophilic compounds [27,38–42]. In-vitro and in-vivo pharmacological and genetic evidence has reported that H_2O_2 [27,40,43], hypochlorite (OCl^-), $\text{O}_2^{\bullet-}$, [39,44], nitric oxide [40,45,46], ONOO^- [40], acrolein, 4-HHE, 4-ONE, and 9-OA- NO_2 , [27,29,38, 42,47] cyclopentenone PGs and iso-PGs have been recognized as TRPA1 activators [41,48]. Finally, hydrogen sulfide (H_2S), the malodorous gas produced by cysteine metabolism, can be included within the list of endogenously produced agonists of TRPA1 [49,50]. There is large body of evidence that oxidative stress by-products generated by chemotherapeutic agents, such as oxaliplatin, paclitaxel, or bortezomib, targeting TRPA1, produce a prolonged condition of mechanical hyperalgesia and cold allodynia in mice, that is reminiscent of the chemotherapeutic-induced peripheral neuropathy observed on humans [51,52, 53[¶]]. This same mechanism may contribute to prolonged airway neuronal hypersensitivity and neurogenic inflammation following exposure to endogenous or exogenous reactive TRPA1 agonists.

ENVIRONMENTAL IRRITANTS AND TRPA1

A rather broad group of environmental irritants has also qualified as TRPA1 channel agonists. Several thousand deaths were sudden, or in the following years, caused by an environmental disaster in Bhopal, India due to a leak of methyl isocyanate (MIC), a precursor in pesticide production [54]. Airway hyperresponsiveness, oedema, injury, and inflammation are reported in individuals exposed to MIC [54]. Additional strong irritants that cause

asthma-related symptoms upon repeated exposure [55] are bifunctional isocyanates, including 2,4-toluene-diisocyanate, diphenylmethane-4,4'-diisocyanate (MDI), and hexamethylene-diisocyanate, which are used in the production of polyurethane products. Tear gases contain 2-chlorobenzylidene malononitrile, and dibenzo[b,f] [1,4] oxazepine, which is the lachrymatory principle present in the currently used riot control agent, and other agents including acrolein, chloropicrin, bromoacetone, and benzyl bromide. Tear gas exposure may result in severe adverse reactions (including acute bronchospasm, pulmonary edema, asthma-like symptoms, severe contact dermatitis, and even death) [56]. These responses may occur more easily in individuals affected by preexisting allergic conditions, which may facilitate an increased reaction after tear gas exposure. Reactive airways dysfunction syndrome (RADS) depicts a clinical condition that develops in some individuals as a consequence of even a single massive exposure to an irritant, gas, vapour or fume, and is characterized by asthma-like symptoms such as cough, wheezing, chest tightness, dyspnoea, airway hyperresponsiveness, and heightened sensitivity to chemical and physical stimuli, including the initial sensitizing stimulus [57]. Work-related asthma encompasses some airway inflammatory conditions caused by exposure to occupational agents [58]. Other related conditions include sensory hyperreactivity and multiple chemical sensitivity [59,60]. Chronic cough and continuous airway hyperresponsiveness may more easily occur if exposure to irritant molecules is prolonged or repeated, and genetic and/or host factors may determine the susceptibility of developing and maintaining RADS [61,62].

Exposure to hypochlorite derives from domestic or industrial accidents, from the weapon gas chlorine, or can be produced by macrophages or neutrophils during oxidative stress. H₂O₂ is a

major component of photochemical smog, vehicle exhaust, tobacco smoke, and smoke from fires and one major component of oxidative stress. Acrolein is an α,β -unsaturated aldehyde and highly reactive molecule contained in tear gas, vehicle exhaust, and produced by peroxidation of plasma membrane phospholipids. Thus, the same reactive agent may be airborne or generated endogenously within the respiratory tissue, but irrespective of the source, it invariably hits its molecular targets. A remarkable number of agents associated with RADS or work-related asthma have been identified as TRPA1 agonists [38,39,47], thereby producing irritation and airway neurogenic inflammation. These include acrolein [63], H₂O₂, and hypochlorite [39,40]. TRPA1 is targeted by MIC and other tear gases [43]. TRPA1 agonists, such as those contained in cigarette smoke, are also produced in the environment, and ozone is a major airborne pollutant among those able to target TRPA1 (see below and Table 1 [27,38–40,43,47,63,64[■],65–67]).

MODELS OF AIRWAY IRRITATION AND INFLAMMATION ACTIVATED BY TRPA1

In a classical model of asthmatic airway inflammation in mice, pharmacological blockade or genetic ablation of the TRPA1 channel markedly reduced cellular infiltration in the airway tissue and bronchoalveolar lavage and hyperresponsiveness to methacholine, a landmark of human asthma [68]. However, TRPV1 deleted mice did not show any reduced inflammatory response in the same allergic paradigm [68]. This observation emphasizes the unique role of endogenously produced pro-inflammatory mediators that target TRPA1 in the underlying mechanism of a mouse model of asthma. The possibility that oxidative stress byproducts generated during asthma exacerbation [69] target TRPA1 to develop an asthmatic phenotype in

Table 1. Toxic inhalation hazards targeting TRPA1 on sensory neurons involved in work-related pulmonary inflammatory diseases

Agent	Toxicant	Syndrome
Photochemical smog, cigarette smoke, automobile exhaust, smoke from fires, tear gas	α,β -Unsaturated aldehydes (crotonaldehyde, acrolein)	RADS, aggravation of asthma, COPD [47,63,64 [■]]
Photochemical smog, cigarette smoke	Saturated aldehydes (formaldehyde, acetaldehyde)	Aggravation of asthma, COPD [65,66]
Photochemical smog, cigarette smoke	ROS, RNS, ozone, hydrogen peroxide	Aggravation of asthma, COPD [27,39,40,47]
Nitration of oleic acid	Nitrooleic acid	Aggravation of asthma, COPD [38]
Occupational exposure	Isocyanates, diisocyanates	RADS, occupational asthma [43,67]
Occupational exposure	Chlorine	Occupational asthma [39]

COPD, chronic obstructive pulmonary disease; RADS, reactive airways dysfunction syndrome; ROS, reactive oxygen species; RNS, reactive nitrogen species.

animal models and human remains an appealing hypothesis that requires, however, further experimental and clinical proof.

Exposure to increased levels of ozone is associated with asthma exacerbation [70]. Noticeably, in humans, ozone exposure evokes airway inflammation, change in airway reactivity, and nociceptive change in lung function [71]. The recently discovered ability of ozone to target TRPA1 [72] may explain all the effects promoted by TRPA1 in the airways of healthy volunteers and their exaggeration in asthma patients [73]. Although these findings have not always been confirmed [74], there is an extensive body of evidence that the use of paracetamol in pregnancy and early infancy is associated with a modest, but consistent, increased prevalence of asthma in late childhood [75,76]. The underlying mechanism that should promote the predisposition to develop asthma after repeated paracetamol exposure is unknown. However, it has been recently reported that N-acetyl-p-benzoquinone imine (NAPQI), the reactive paracetamol metabolite, which, when the drug is overdosed, causes life-threatening liver injury, may be produced in mouse lung at therapeutic doses (even those used in children) [77[¶]]. The finding that NAPQI is a powerful TRPA1 agonist and by this mechanism paracetamol produces transient and moderate, but repeated, neurogenic inflammation episodes in the airways, has led to the hypothesis that this mechanism contributes to the transition of susceptible individuals to an asthmatic phenotype [77[¶]]. The major causative factor of COPD is the cigarette smoke habit. Cigarette smoke contains more than 5000 constituents, and many of them, which exhibit irritant properties, have been characterized as TRPA1 agonists. These include, among others, the unsaturated aldehydes crotonaldehyde and acrolein [64^{¶¶},78], the saturated aldehydes formaldehyde [65] and acetaldehyde [66], and ROS, such as H₂O₂ [39,40]. The early neurogenic inflammatory response, produced in rodent airways after inhalation of cigarette smoke, is now known to be entirely mediated by TRPA1, expressed by vagal primary sensory neurons [64^{¶¶}].

TRPV1 stimulation after agonist inhalation mediates a cough response that appears to be exaggerated under a series of circumstances, including airway inflammation [79,80]. More recently, TRPA1 agonists have been shown to evoke cough in guinea pigs [78] and in healthy humans [81[¶]]. However, whereas the TRPV1-dependent cough reflex was enhanced in current smokers, this was not the case for TRPA1-dependent cough [82]. The role of TRPV1 and TRPA1 in chronic cough conditions, as in post-viral cough or during RADS, has not been explored.

CONCLUSION

Activation of TRP channels, mainly TRPV1 and TRPA1, in sensory nerve endings of the respiratory system has been reported to produce irritation, activation of protective reflex responses (cough), and neurogenic inflammation. Such functions may be activated through TRPA1 stimulation by a large array of molecules, including those present in air pollution and in the work place, inhaled as a recreational habit, or endogenously produced under inflammatory conditions, as in asthma [69] or COPD [83]. Thus, occupational airway inflammation may recognize two principal mechanisms to activate a common final pathway represented by TRPA1. Electrophilic and reactive compounds may derive from exogenous sources or may be produced endogenously during the inflammatory process. The level of interaction or synergy between these two pathways and the relative impact on disease progression is at present unknown. Another key issue that should be considered is the recent identification of extraneuronal TRPA1 in airway and lung tissue. TRPA1, first cloned in human lung fibroblasts, has been identified in airway and lung fibroblasts and epithelial cells and bronchial smooth muscle cells, where it stimulates interleukin-8 (IL-8) release [84,85[¶]]. TRPV1 channels appear to be expressed by human airway epithelial cells and overexpressed in the airways of patients with refractory asthma, where they release IL-8 [86[¶]]. The significance of extraneuronal TRPs in airway diseases remains to be determined, but it is possible that such localization and function may open new avenues for the understanding and treatment of a variety of diseases of the respiratory system. Endogenously formed TRPA1 agonists may be principally important in asthma and COPD. Inhaled irritants that target TRPA1 may offer new clues for the understanding and treatment of the various forms of work-related asthma. Finally, extraneuronal TRPV1 and TRPA1 may contribute with additional non-neurogenic inflammation to the previously reported diseases, and perhaps to more severe conditions such as pulmonary edema and adult respiratory distress syndrome (ARDS) seen with exposure to toxic inhalation hazards [87].

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Conflicts of interest

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- of special interest
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