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Role of TRPV Receptors in Respiratory Diseases

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Abstract

Transient receptor potential vanilloid type channels (TRPVs) are expressed in several cell types in human and animal lungs. Increasing evidence has demonstrated important roles of these cation channels, particularly TRPV1 and TRPV4, in the regulation of airway function. These TRPVs can be activated by a number of endogenous substances (hydrogen ion, certain lipoxygenase products, etc.) and changes in physiological conditions (e.g., temperature, osmolarity, etc.). Activation of these channels can evoke Ca^{2+} influx and excitation of the neuron. TRPV1 channels are generally expressed in non-myelinated afferents innervating the airways and lungs, which also contain sensory neuropeptides such as tachykinins. Upon stimulation, these sensory nerves elicits centrally-mediated reflex responses as well as local release of tachykinins, and results in cough, airway irritation, reflex bronchoconstriction and neurogenic inflammation in the airways. Recent studies clearly demonstrated that the excitability of TRPV1 channels is up-regulated by certain autacoids (e.g., prostaglandin E_2 , bradykinin) released during airway inflammatory reaction. Under these conditions, the TRPV1 can be activated by a slight increase in airway temperature or tissue acidity. Indirect evidence also suggests that TRPV channels may play a part in the pathogenesis of certain respiratory diseases such as asthma and chronic cough. Therefore, the potential use of TRPV antagonists as a novel therapy for these diseases certainly merits further investigation.

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1. Introduction

Transient receptor potential (TRP) ion channel family consists of 28 ion channels, and can be further divided into six subgroups based upon the structure and activation characteristics of the channels [1, 2]. TRP subfamilies include canonical (TRPC, 7 channels), melastatin (TRPM, 8 channels), ankyrin (TRPA, 1 channel), vanilloid (TRPV, 6 channels), polycystin (TRPP, 3 channels) and mucolipin (PRTML, 3 channels) families. TRPV channel family (TRPV1-6) are widely expressed in various cell types of the mammalian organ systems. Since these TRPV channels were cloned in the last several years, increasing evidences begin to reveal the important physiological properties and function of these channels. TRPV channels are nonselective cation channel with a high permeability to Ca^{2+} , and the selectivity of cations varies among different TRPV channels. It is well recognized that intracellular calcium is an important signal transduction molecule in all cells, and plays a critical role in the regulation of membrane excitability, neurotransmitter release, synaptic transmission and other important biological functions in neurons. Hence, because of the high permeability to Ca^{2+} and their sensitivities to various physiological stimuli, TRPV channels are believed to play an important role in the regulation of airway function under both normal and disease conditions.

Ion channels in TRPV family can be activated by a diverse range of biological stimuli that are found in the respiratory tract, including acid, changes in temperature and osmolarity, mechanical stress, change in intracellular Ca^{2+} concentration, and various inflammatory mediators. In addition, up-regulation of TRPV expression and sensitivity in

the lung may also contribute to the manifestation of various symptoms of airway inflammatory diseases. In this review, we discuss the expression and function of TRPV channels in the lung, the endogenous substances and physiological conditions that can activate and modulate the sensitivity of these channels, and the potential involvements of TRPVs in the pathogenesis of certain respiratory diseases.

2. TRPV channel expression and function in the lung

Several TRPV channels have been detected in mammalian lung tissues, including airway sensory nerves, airway smooth muscle cells, epithelial cells, vascular endothelial cells, submucosal glands, and inflammatory cells [3-7]. Although their precise roles in regulating the function of each individual cell type are not clearly defined, recent studies have revealed some convincing evidence of their involvements under both normal and disease conditions. Of course, many of these studies were performed in the heterologously expressed TRPV channels, cautions are required in extrapolating these findings to the responses in native cells and biological systems, and certain inherent limitations should also be taken into consideration in predicting the potential physiological implications. We will focus this review primarily on the function of TRPV1 and TRPV4 expressed in the respiratory tract because of the recent progresses in research on these two TRPV channels and our existing knowledge about their possible involvements in the pathogenesis of certain airway diseases.

2.1 TRPV1

TRPV1 expression in the airway: The expression of the TRPV1 in the mammalian respiratory tract is predominantly localized in the sensory nerves, despite that its presence in other cell types has also been reported. The afferent activities arising from sensory terminals located in the lung and airways are conducted primarily by branches of vagus nerves [8, 9] that project to the nucleus tractus solitarius in the brainstem, and their cell bodies reside in the nodose and intracranial jugular ganglia. Among these vagal bronchopulmonary afferents, approximately 75% are small-diameter, slow-conducting non-myelinated (C-) fibers [10]. One of the most distinct characteristics of these C-fiber afferents is the functional expression of TRPV1 at the sensory terminals, as evident by their exquisite sensitivity to capsaicin delivered either by inhalation or by injection into the pulmonary circulation [11, 12]. In a sharp contrast, their myelinated counterpart of the vagal pulmonary afferents either do not or rarely exhibit any sensitivity to capsaicin [11].

Immunohistochemical evidence shows that TRPV1 channels are often co-localized with certain sensory neuropeptides, such as tachykinins (TKs) and calcitonin gene-related peptide (CGRP), in the same axon [7, 13, 14]. These TRPV1-expressing C-fiber endings also display distinct sensitivity to chemical irritants as well as certain endogenously released autacoids [11, 12, 15, 16]. Recent studies suggest that the actions of some of these stimulants are mediated through the activation of TRPV1. These chemosensitive endings innervate the entire respiratory tract, from upper airways (nose, larynx, trachea) to lung parenchyma (alveolar wall). They are found in the mucosa as well as in the deeper regions of the airway structure (e.g., near smooth muscles). In the conducting airways, they show extensive axonal arborization that either extends into the space between epithelial cells or

forms network-like plexus immediately beneath the basement membrane of airway epithelium in various species including humans [17-19].

Biological response to TRPV1 activation: Activation of TRPV1 channels on airway sensory nerves induces Ca^{2+} influx into the neuronal cell, membrane depolarization and action potentials. Activation of these TRPV1-expressing vagal sensory terminals in the airways can elicit extensive reflex responses such as bronchoconstriction, mucus secretion, bradycardia and hypotension mediated through the efferent pathways of the autonomic nervous system, which are accompanied by rapid shallow breathing, airway irritation and cough. Together, these are the typical respiratory defense responses against inhaled irritants [12, 20]. Ca^{2+} influx evoked by TRPV1 activation can also trigger the release of TKs and CGRP from both peripheral and central nerve terminals. These neuropeptides are known to act on a number of effector cells in the respiratory tract (e.g., airway and vascular smooth muscles, cholinergic ganglia, inflammatory cells, mucous glands), and cause bronchoconstriction, protein extravasation, airway mucosal edema and inflammatory cell chemotaxis [21, 22]. Thus, prolonged and intense stimulation of these endings can lead to the development of "neurogenic inflammatory reaction" in the airways [21, 23, 24].

2.2 TRPV4

TRPV4 expression in the lung: The TRPV4 channel is widely expressed in mammalian tissues including lung, heart, kidney, sensory neurons, sympathetic nerves, brain, skin, intestine, salivary gland, sweat glands, inner ear, endothelium and fat tissue [25-30]. In the lung, TRPV4 is detected by RT-PCR in a human bronchial epithelial cell

line [31] and in cultured human airway smooth muscle cells [32]. Immunohistochemistry study showed that TRPV4 is expressed in the alveolar wall in human, rat and mouse lung [33].

Biological response to TRPV4 activation: Activation of TRPV4 channel increases intracellular Ca^{2+} concentration and thus may play an important role in Ca^{2+} signaling during osmotic stimulation. The physiological role of TRPV4 activation may vary among different tissues. TRPV4 in human bronchial epithelial cells may be involved in hypotonic solution-induced cell regulatory volume-decrease response through the activation of Maxi K^+ channels [31]. TRPV4 activation also induces Ca^{2+} influx in human airway smooth muscle cells as well as smooth muscle contraction in isolated human and guinea pig airways [32]. In isolated rat lung, TRPV4 activation increases endothelial permeability and disrupts the alveolar septal barrier [33].

3. Endogenous TRPV activators and modulators in normal and disease conditions

3.1 Endogenous activators of TRPV1

Recent studies have demonstrated several endogenous chemical substances that can activate TRPV1 in the lung, and their stimulatory effects become more pronounced under various pathophysiological conditions in the airways.

Hydrogen Ion: Inhalation of acidic aerosol or right-atrial injection of lactic acid elicits cough and reflex bronchoconstriction in humans and animals [34-36], suggesting that stimulation of lung afferents is involved [37, 38]. It is known that hydrogen ion can modulate the stimulatory effect of capsaicin on heterologously expressed TRPV1 [39].

The sites of interaction have been suggested to be located within putative extracellular loops of TRPV1, particularly at two glutamate residuals E648 and E600 [40]. The evidence of acid stimulation of TRPV1 in the airway nerves was first reported by Fox and co-workers [41] who demonstrated that C-fiber afferents innervating the guinea pig trachea were stimulated when the isolated guinea pig airway was perfused with acidic buffer at pH of 5.0, and that the effect was abrogated by capsazepine [42]. However, it is also possible that certain autacoids (e.g., lipooxygenase metabolites, etc.) are released from the surrounding tissue upon the action of hydrogen ion, which in turn can activate the TRPV1 receptor on the nerve terminals [43]. A direct stimulatory effect of hydrogen ions on TRPV1s was recently demonstrated in pulmonary sensory neurons isolated from rat nodose/jugular ganglia [44]. The current evoked by lowering the pH of extracellular solution to 7.0 consisted of only a transient, rapidly inactivating component with small amplitude, which increased in amplitude when the H⁺ concentration was elevated. In addition, a slow, sustained inward current began to emerge when pH was reduced to below 6.5. The transient component was dose-dependently inhibited by amiloride, a common blocker of acid-sensing ion channels (ASICs) [45], whereas the sustained component was almost completely abolished by capsazepine, indicating a critical involvement of TRPV1 in the acid-evoked current in these neurons (Fig. 1) [44]. Presumably, this long-lasting effect of TRPV1-activation is responsible for, to some extent, the lingering irritant effect of acid on the airways.

Lipoxygenase Products of Arachidonic Acid: Products derived from lipoxygenase metabolism of arachidonic acid are released in inflammatory tissues, and are believed to be involved in the inflammation-induced hyperalgesia [46]. Some of these lipoxygenase

products, such as 12-(S)- and 15-(S)-hydroperoxyeicosatetraenoic acid (12S- and 15S-HPETE), have been shown to exert a highly potent stimulatory effect on TRPV1 [43], and several isoforms have been found in the lung tissue [47-49]. These lipoxygenase products are known to be released in the airways during asthmatic attack [50], and therefore are considered as the primary candidates of the endogenous TRPV1 activators in airway inflammatory diseases. However, despite all the suggestive evidence, the convincing in-vivo or in-vitro evidence demonstrating the direct activation of TRPV1-expressing sensory nerves in the airways is still lacking; this discrepancy may be related to the instability of these compounds and the difficulty of effectively delivering them to the receptor site. This notion is supported by the findings that endogenously released lipoxygenase products are critically involved as the intermediate mediators in the activation of TRPV1 by certain autacoids (e.g., bradykinin) [51-53].

Anandamide: Anandamide, an endogenous neuronal lipid mediator, is the ethanolamine amide of arachidonic acid first isolated from porcine brain [54]. Anandamide is synthesized in the nervous system as well as in peripheral tissues including airways and lung [55, 56]. Anandamide is previously known as a cannabinoid receptor agonist. Recent evidence shows that anandamide also activates human and rat TRPV1 receptors in TRPV1-transfected cells, rat dorsal root ganglia (DRG) neurons in vitro [57, 58], and bronchopulmonary C-fiber afferents in vivo [59-61]. Anandamide has a similar K_i for TRPV1 in recombinant cell lines, but a distinctly lower potency in various assays [62], which raises the question as to the probability of its action as an endogenous activator of TRPV1 [61]. Anandamide is found to be either a partial or a full agonist depending on the tissue tested as well as the physiological conditions. For

example, as a partial agonist, anandamide may sensitize the channel response to other TRPV1 agonists, while in certain pathophysiological conditions (e.g., airway inflammation) when TRPV1 sensitivity is up-regulated, anandamide may serve as an endogenous agonist and activate the channel. A recent study further revealed the role of anandamide as an intracellular messenger that is formed following the activation of phospholipase C / inositol 1,4,5-triphosphate pathway and can activate the TRPV1 in rat DRG neurons [62].

Hyperthermia: TRPV1 is generally considered as the sensor for detecting noxious heat in the somatic sensory system, and its response characteristics and channel activation by heat are well documented [63, 64]. Because the temperature threshold for its activation is relatively high (43°C) and the pulmonary sensory nerves is constantly exposed to body temperature, a possible role of the TRPV1 as a thermal sensor in the physiological range of body temperature was generally overlooked. However, a recent study showed that increasing temperature from 23°C to 41°C in a ramp pattern evoked an inward current or membrane depolarization in isolated rat pulmonary sensory neurons after exceeding a threshold of ~34.4°C. Further increase in temperature increased the amplitude of inward current sharply, or evoked action potential as the temperature reached 39-40°C. The temperature coefficient, Q_{10} , was 29.5 ± 6.4 over the range of 35–41°C and distinctly higher than that over the lower temperature range (23–30°C, $Q_{10} = 2.84 \pm 0.56$), which clearly illustrates the thermal sensitivity of these pulmonary sensory neurons [4]. Approximately 48% of the response was blocked by pretreatment with capsaizepine, suggesting an important role of the TRPV1; the remaining response probably involves TRPV2-4 channels because it was totally abolished by ruthenium red,

a non-selective TRPV and calcium channel blocker (Fig. 2) [4]. In view of the facts that hyperthermia occurs commonly in normal (e.g., during exercise) as well as in disease conditions (e.g., fever, heat stroke) and that a significantly higher tissue temp was found in the airways of asthmatic patients [65], the thermal sensitivity of individual TRPV channels and their relative contributions to the regulation of airway function certainly require further investigation.

Other endogenous TRPV1 activators: Based upon the structure-activity relationship studies, several endogenous agonists of TRPV1 have been proposed. *N*-arachidonoyl-dopamine (NADA) is an endogenous substance with structural similarity to capsaicin and has been found in mammalian nervous tissues, mostly in the central nervous system. NADA activates the TRPV1 over-expressed in HEK293 cells, with potency and efficacy similar to those of capsaicin. Furthermore, NADA can activate TRPV1 channels in the nociceptive neurons in rat dorsal root ganglion [66]. Similarly, *N*-oleoyldopamine (OLDA) was found in mammalian brain, and can activate the TRPV1-transfected HEK cells in vitro and induce a hyperalgesic effect in vivo [67], which can be blocked by iodo-resiniferatoxin, a TRPV1 antagonist. To our knowledge, the physiological and pharmacological actions of these compounds on capsaicin-sensitive afferents innervating the airways and lung have not been studied.

Some interesting studies recently reported that increasing extracellular concentrations of cations, such as Na^+ , Mg^{2+} , and Ca^{2+} , can actively gate the TRPV1 channel expressed in HEK293 cells and oocytes, and these effects seem to result from electrostatic interactions between these ions and two glutamates located near the channel pore (E600 and E648), formerly identified as proton-binding residues [40, 68]. In

addition, polyamines, such as spermine and putrescine, which are positively charged can directly activate TRPV1 in a charge-dependent manner, both in heterologous expression systems and in sensory neurons [69]. These observations are consistent with the finding that eosinophil granule-derived proteins carrying cationic charges can activate and sensitize the TRPV1-expressing pulmonary sensory nerves in anesthetized rats [70, 71].

Reactive Oxygen Species (ROS) such as hydrogen peroxide and hydroxyl radicals have been suggested to play a part in the nociceptor activation during tissue inflammation and ischemia [72], but transduction mechanism was not fully understood. Recent studies have revealed that activation of the TRPV1 receptor by cyclooxygenase metabolites is partially responsible for ROS-evoked stimulation of pulmonary C-fiber afferents in rats because the stimulatory effect of aerosolized hydrogen peroxide was reduced to half by pretreatment with either capsazepine or indomethacin [73, 74].

Although most of the TRPV channels are considered to be voltage independent, a recent study has clearly shown that physiological stimuli of TRPV1 (and some other temperature-sensitive TRP channels), such as high temperature and capsaicin, can shift its activation curve (open probability vs. voltage) from a non-physiological positive voltage range towards the negative potential [75]. This large shift of voltage-dependent activation curve to a physiologically relevant voltage range with a relatively small gating charge is believed to be an important factor underlying the functional properties of TRPV1 and its sensitivity to a diverse range of biological stimuli [75].

3.2 Endogenous TRPV4 activators

Heterologously expressed TRPV4 channel can be activated by hypotonic solutions [27], phorbol derivatives [76, 77] and by increase in temperature [25, 78]. It has been reported that the osmolarity of airway surface fluid is about 80% of isotonic body fluids (222 vs 285 mOsM) in healthy humans [79]. However, the osmolarity of airway surface fluid is significantly decreased in certain airway diseases (e.g., asthma; [79]). Hypotonicity has also been reported in rat and mouse airway surface fluid [80, 81]. This level of hypotonicity is likely to be sufficient to activate TRPV4 if airway smooth muscle cells are exposed in situ. In normal airways, TRPV4 on smooth muscle cells may not be activated by the hypotonic airway surface fluid because of the epithelium barrier. However, when the epithelium is disrupted in inflammatory airway diseases such as asthma [82, 83], airway smooth muscles are exposed to the hypotonic airway surface fluid, which may then activate the TRPV4 on these muscle cells. Indeed, inhalation of distilled water or hypotonic saline aerosols induces bronchoconstriction and a fall in forced expiratory volume in one second (FEV1) in asthma patients but not in normal subjects [84-88]. Part of the bronchoconstriction is probably mediated through cholinergic reflex since hypotonicity can stimulate both C-fiber afferents and rapidly adapting receptors in the airways, although a lack of chloride ion appears to be also a contributing factor [89-91]. TRPV4 activation also increases the permeability of endothelial cells and disrupts the alveolar septal barrier in isolated rat lung. Therefore, it has been suggested that the TRPV4 activation may be involved in the development of acute lung injury [33].

TRPV4 can be also activated by increase in temperature with an activation threshold of 24° -32°C [2, 25, 78, 92], which is below the normal range of body

temperature. Thus, whether the thermal sensitivity of TRPV4 plays any role in the regulation of physiological function of lung afferents remains to be determined, despite that both mRNA and protein expression of TRPV4 have been demonstrated in the pulmonary sensory neurons [4].

3.3 Endogenous modulators of TRPV sensitivity

It is well documented that a number of endogenous inflammatory mediators can modulate the sensitivity of TRPV1 during tissue inflammation, which leads to nociceptor hypersensitivity and hyperalgesia. The mechanisms underlying the sensitization of TRPV1 are not yet fully understood, but several signal transduction pathways are known to be involved; TRPV1 has several consensus phosphorylation sites that can be phosphorylated by protein kinases A, C, and G (PKA, C and G), tyrosine kinase, etc. [93-96]. As demonstrated in recent studies, some of the endogenous mediators can modulate the excitability of TRPV1 expressed in pulmonary/airway sensory neurons and enhance the airway responsiveness to chemical irritants.

Prostaglandin E₂: The sensitivity of cough reflex elicited by capsaicin is enhanced by inhaled PGE₂ in healthy human volunteers [97], suggesting a PGE₂-induced sensitization of TRPV1 in pulmonary sensory nerves. Indeed, this contention is supported by the observation that exogenous PGE₂ at a low dose markedly enhances the excitabilities of vagal pulmonary C fibers to capsaicin in anesthetized rats [98]. In cultured nodose and jugular pulmonary sensory neurons, PGE₂ (1 μM) markedly increased the whole cell current density and the number of action potentials evoked by capsaicin [94]. The sensitizing effect of PGE₂ could also be mimicked by butaprost [94], a selective agonist of the EP₂ prostanoid

receptor, or by a direct activation of adenylyl cyclase or protein kinase A (PKA), and prevented by pretreatment with inhibitors of adenylyl cyclase or PKA [93]. Taken together, these studies suggest that PGE₂ activates the G_s protein-coupled EP₂ prostanoid receptor present on the membrane of these neurons, which upon activation increases the enzyme activity of adenylyl cyclase [93, 94, 99]. The resulting rise in cAMP may then stimulate PKA, which in turn increases the phosphorylation of TRPV1 and enhances its excitability [94, 100].

Bradykinin: Bradykinin, a nonapeptide derived from kininogen precursors, is produced upon the activation of tissue or plasma kallikreins by tissue damage, viral infection and other inflammatory reactions. A pronounced increase in kinin and kallikrein activity in the bronchoalveolar lavage were found in patients suffering from asthma attack or anaphylaxis [101]. Bradykinin has been shown to enhance the sensitivity of C-fiber afferents to capsaicin in isolated guinea pig airways [102]. Although the mechanisms underlying this action are still not fully understood, activation of the G protein-coupled bradykinin B₂ receptors is believed to play a part in this action. An involvement of TRPV1 channel in the bradykinin-induced increase in the excitability of these sensory neurons has been reported in several recent studies [60, 95, 103]. One possibility is that modulation of the TRPV1 current by bradykinin is generated by a direct action of PKC on the TRPV1 receptor [95]. A recent study by Carr et al. [51] further revealed that B₂ receptor-mediated effect of bradykinin on airway C fibers is mediated through the production of lipoxygenase products that in turn activate TRPV1 receptors. This possibility is supported by the finding that bradykinin induces the production of 12-lipoxygenase metabolites of arachidonic acid in DRG neurons [53]. Furthermore, activation of the B₂ receptor is known to stimulate phospholipase C and

increase production of diacylglycerol (DAG), which in turn activates the protein kinase C (PKC) [103]; pretreatment with the B₂-receptor antagonist or the PKC inhibitor effectively attenuates the effect of bradykinin on these nociceptive afferents [104-106]. In isolated guinea pig trachea-bronchus preparation, bradykinin has been shown to activate airway C-fiber afferents through a combined effect of activation of both TRPV1 and Ca²⁺-activated chloride channels [107].

Nerve Growth Factor (NGF): NGF is a member of neurotrophin family, and known for its important influence on the growth and maintenance of certain specific subsets of peripheral sensory neurons [108]. Recent investigations have further revealed its important role as an inflammatory mediator during acute tissue injury; in patients with asthma or other types of airway inflammatory reactions, there is a pronounced increase in the NGF level in the serum and bronchoalveolar lavage fluid [109, 110]. Administration of NGF in somatic tissues induces both acute and long-lasting hyperalgesic effects, and increases the capsaicin sensitivity in DRG nociceptive neurons [111]. In cultured DRG neurons or frog oocytes co-expressing TRPV1 and TrkA tyrosine kinase receptors, NGF increases the neuronal sensitivity to capsaicin, hydrogen ion and thermal stimulation [103, 111]; the responses to these stimuli seem to be linked specifically to a modulatory effect on the TRPV1 [103]. Whether NGF also alters the excitability of other ligand-gated channels in nociceptive neurons is unclear [112]. This hyperalgesic action of NGF on DRG neurons is believed to be mediated through the G-protein-coupled TrkA receptors, which in turn activates mitogen-activated protein kinase and phospholipase C (PLC)- γ signaling pathway [103, 113]. Furthermore, these studies suggest that PLC activation is the primary pathway in the NGF-induced potentiation of TRPV1 channel

[103]. A recent study further demonstrated that phosphoinositide 3-kinase (PI3K) is physically and functionally coupled to TRPV1, which facilitates trafficking of TRPV1 to the plasma membrane [114]. More interestingly, the NGF-induced sensitization of DRG neurons can be completely abrogated by inhibition of PI3K activity [114].

Hydrogen Ion: It is well documented that pH is reduced in the extracellular fluid of inflamed and ischemic tissues. In patients during asthmatic attack, the pH in the expired breath condensate is reduced to 5.23, as compared to 7.65 in healthy subjects. This abnormally low airway pH returns to normal after anti-inflammatory therapy, suggesting that the airway acidosis is caused by tissue inflammation [115, 116]. Tissue acidification has been shown to induce hyperalgesia by sensitizing nociceptors in the somatic afferents [117, 118]. Electrophysiological recording further demonstrated that acid enhanced the stimulatory effect of capsaicin on DRG neurons and heterologously expressed TRPV1, indicating a positive interaction between H^+ and TRPV1 [39, 118, 119]. Acidification of lung tissue occurs commonly when CO_2 concentration increases in the alveolar gas, resulting from exceedingly high CO_2 production (e.g., during strenuous exercise) and/or from hindered CO_2 elimination from the lungs (e.g., in obstructive airway diseases). A recent study showed that the stimulatory effect of capsaicin on pulmonary C-fiber afferents was markedly elevated, when pH in the pulmonary venous (left atrial) blood was reduced from 7.40 to 7.17 during alveolar hypercapnia [120]. The hypersensitivity of TRPV1 could be largely prevented by infusion of bicarbonate to minimize the change in blood pH, further demonstrating the modulatory effect of H^+ on TRPV1 sensitivity.

Proteases: Proteases such as trypsin and elastase are released from mast cells and leukocytes during tissue inflammation and injury, and can cleave protease-activated receptor 2 (PAR2) expressed on primary afferent neurons to cause neurogenic inflammation and hyperalgesia in somatic tissue. The PAR2-induced hyperalgesic effect involves sensitization of TRPV1, and can be abrogated by antagonist of PAR2, PKC or TRPV1 [121, 122]. In DRG neurons, stimulation of PAR2 activates PKC ϵ and PKA, which in turn increases the TRPV1 excitability [123]. A recent study showed that activation of PAR2 up-regulates the excitability of isolated rat pulmonary chemosensitive neurons, and the effect of PAR2 activation was mediated through the PLC/PKC transduction pathway [44]. These results suggest that the interactions between PAR2 and pulmonary sensory nerves may play a part in the airway hyperresponsiveness induced by PAR2 activation. Indeed, it has been reported that PAR2 activation increases the TRPV1-mediated cough in guinea pigs via activation of PKC- and PKA-signal transduction pathways as well as the endogenous release of prostanoids [124]. A recent report further demonstrated that PAR2 cleavage by proteases activates a second messenger to increase the sensitivity of the TRPV4 naturally expressed in the bronchial epithelial cells and induce mechanical hyperalgesia in mice [125].

4. Potential role of TRPV channels in respiratory diseases

4.1 Cough

Cough is arguably the most common symptom associated with pulmonary diseases, such as bronchitis, COPD and the common cold. This respiratory defense

mechanism is facilitated by a coordinated effort of neuromuscular elements that comprises a complex reflex arc. The reflex arc includes a vagal sensory afferent limb, a central processing center located in the lower brainstem referred to as the “cough center” and an efferent motor limb. Increasing evidence has suggested a significant role of TRPV1 in the genesis of cough, as evidenced by the following observations. Firstly, it is generally believed that the airway sensory nerves expressing TRPV1 receptors are involved in eliciting the cough reflexes [100, 126]. Inhalation of capsaicin or acid aerosol consistently and reproducibly produces coughs in a dose-dependent manner in humans and various animals [34, 127-129], and both these tussive agents commonly used in human studies are potent TRPV1 activators. Secondly, a number of endogenous inflammatory mediators that are known to upregulate the TRPV1 sensitivity, such as PGE₂, bradykinin, histamine, etc., can also enhance the cough sensitivity in experimental animals as well as in human subjects [97, 102, 130]. Thirdly, over-expression of TRPV1 is found in the bronchial tissue of patients with chronic cough [131, 132]; when the airway mucosal biopsies were obtained by fiberoptic bronchoscopy and compared between healthy volunteers (n=17) and patients with chronic cough (n=29), a five-fold increase of TRPV-1 immunoreactive nerve profiles was found in patients with cough [131] (Fig. 3). Furthermore, there was a significant correlation between the cough sensitivity to capsaicin inhalation challenge and the density of TRPV1-expressing nerves in the mucosa of patients with cough (Fig. 3). These findings offer compelling evidence to suggest that an increase in expression and/or sensitivity of TRPV1 in the sensory endings of airway mucosa may be involved in the development of chronic cough.

4.2 Asthma

Asthma is a chronic inflammatory disease characterized by reversible airway obstruction and bronchial hyperresponsiveness to various stimuli, and indirect evidences have suggested a potential involvement of TRP channels in the pathogenesis of asthma. For example, it is known that TRPC6 is expressed in eosinophils, neutrophils, mast cells and CD4⁺ T-lymphocytes, the major immune cells involved in the pathogenesis of asthma and airway allergic reaction [133-135]. In addition, expression of TRPV1 in airway C-fiber sensory nerves is well documented, and activation of these sensory nerves by endogenous TRPV1 activators or inhaled irritants and the resulting release of tachykinins are believed to contribute to the manifestation of various pathophysiological features of asthma. Endogenous TRPV1 activators, such as airway acidification and lipoxigenase products, have been detected in asthmatic patients [50, 115]. Inhaled capsaicin induces bronchoconstriction in 40% of asthma patients but not in normal subjects [136]. Capsaicin-induced cough response is increased in asthmatic patients, indicating an increased TRPV1 activity in asthma. In allergic animal models, pre-treatment with capsaicin to degenerate the airway TRPV1-expressing afferents and to deplete sensory neuropeptides, inhibits allergen-induced airway constriction in sensitized guinea pigs [137] and airway hyperresponsiveness in allergic rabbits [138], suggesting an important role of these airway sensory nerves. In a mouse model of non-atopic asthma, dinitrofluorobenzene sensitization and challenge induces inflammatory cells accumulation and airway hyperresponsiveness; these responses can also be inhibited by capsaicin pretreatment [139]. Recent studies have further presented convincing evidence of an important role of TRPV1 in the manifestation of various symptoms of airway

hypersensitivity associated with airway inflammation [16, 32, 140]; for example, bronchomotor response and cough sensitivity to TRPV1 activators, capsaicin or citric acid aerosol, are markedly elevated in patients with asthma or airway inflammation [141, 142]. One of the most prominent pathophysiological features of asthma is bronchial hyperresponsiveness [143, 144], which is generally believed to be closely associated with endogenous release of various chemical mediators from inflammatory cells. Recent studies have demonstrated that some of these inflammatory mediators (e.g., prostaglandins, bradykinin, leukotrienes, eosinophil granule-derived cationic proteins, etc.) can markedly enhance the sensitivity of TRPV1 and lower its threshold for activation [16, 71]. Furthermore, a recent report has further revealed a higher tissue temperature in the airways of asthmatic patients [65]. Thus, a given level of stimulation may evoke a greater afferent discharge of the TRPV1-expressing C-fiber endings and consequently a more severe bronchoconstriction via both central cholinergic pathway and local axon reflex [16].

Exercise-induced asthma is a pathophysiological condition of the airways induced by hyperventilation of dry air, and present in approximately half of the asthmatic patients [145, 146]. It is generally believed that the primary cause of bronchospasm during exercise is the injury of airway epithelium caused by evaporation during hyperventilation, resulting the release of various inflammatory mediators such as leukotrienes and histamine [145, 146], which in turn triggering bronchoconstriction. Recent studies have demonstrated that TRPV4 is not only activated by hypotonicity as described in the original observation [30], but also by a mild increase in osmolarity, especially at the presence of tissue inflammation [147, 148]. However, whether activation of TRPV4 channels expressed in the tracheobronchial epithelial cells and subsequent Ca^{2+} influx are involved in triggering

the release of autacoids from epithelial and inflammatory cells during exercise-induced asthma remains to be determined.

4.3 Chronic Obstructive Pulmonary Disease (COPD)

COPD is an airway disease characterized by chronic inflammation, and airflow obstruction that is usually progressive, and not reversible; the disease state is often associated with the presence of emphysema or chronic bronchitis. To our knowledge, there is no existing evidence indicating a direct link between TRP channels and the pathogenesis of COPD. However, it is known that an increased production of certain endogenous cytokines is involved in the chronic airway inflammation and disease process of COPD [149, 150], and indirect evidence suggests that some of these pro-inflammatory cytokines may lead to the stimulation of TRPV1-expressing lung afferents. For example, tumor necrosis factor α and interleukin 6 (IL-6) have been shown to sensitize the DRG nociceptors and play a part in the inflammatory hyperalgesia [151, 152], and IL-1 β can stimulate pulmonary C-fibers in rats and rabbits [153, 154]. Moreover, certain cytokines are known to cause an increased release of prostaglandins [155], such as PGE₂, which can enhance the sensitivity of TRPV1 in pulmonary sensory neurons [94]. Several types of the proteases released from inflammatory cells such as mast cells and macrophages during the chronic airway inflammation are also known to modulate the TRPV1 sensitivity [44, 124]. In addition, activation of TRPV1 is known to evoke the release of tachykinins from sensory endings in the airways, and these neuropeptides can exert chemotactic action on certain immune cells (e.g., mast cells and T-lymphocytes expressing neurokinin-1 receptors), which

in turn may induce inflammatory reaction and contribute to the progressive deterioration of airway structure and function [21, 156].

4.4 Other airway diseases

Respiratory Viral Infection: Respiratory viral infections are often accompanied by inflammation and injury of the airway mucosa that is densely supplied by tachykinin-containing, TRPV1-expressing sensory nerves [17, 18]. Viral infection is also frequently associated with more serious complications such as chronic cough and exacerbation of asthma, especially in young children [157, 158]. Neurogenic inflammation in the airways evoked by TRPV1 activation with capsaicin was amplified in rats inoculated with respiratory syncytial virus (RSV), resulting from the upregulation of neurokinin-1 (NK-1) receptors in the airway epithelium and vascular epithelium [159]. Stimulation of the TRPV1-expressing sensory nerves is also involved in the overexpression of NK-1 receptors in CD4⁺ T cells and the chemotactic effect on these lymphocytes during the RSV infection in the airways [160]. Furthermore, inoculation of guinea pigs or rats with RSV or Sendai virus (parainfluenza virus) generates long-lasting airway inflammation, promotes over-expression of NGF, and causes a phenotypic switch in tachykinergic innervation of the airways [158, 161, 162]. Whether these observed increases in the airway tachykinin synthesis and expression of NK-1 receptor are accompanied by upregulation of TRPV1 expression in the viral infection-induced airway hyperreactivity [163] is not known. More importantly, whether respiratory viral infection alters the excitability of TRPV1 in the airway sensory nerves also remains to be determined.

Gastroesophageal Reflux Diseases: The association between gastroesophageal reflux and asthma has been well documented in the literature; the prevalence of gastroesophageal reflux symptoms in asthmatics is significantly higher than that in the control population [164]. As described earlier, TRPV1s are found in the unmyelinated sensory nerves innervating the upper airways including larynx and trachea. The pH in the gastric acid can be as low as 2.0; microaspiration of gastric acid can activate these laryngeal and tracheobronchial C-fibers, and elicits reflex bronchospasm via the cholinergic efferent pathway and also evokes local release of tachykinins in the airways. The latter can cause neurogenic inflammation, mucus secretion, bronchospasm and cough. Furthermore, vagal afferents innervating the esophagus can be also activated by capsaicin and low pH [165], suggesting expression of TRPV1 in these sensory nerves. Indeed, a recent study reported that TRPV1-immunoreactive sensory fibers are expressed in human esophagus, and the expression is upregulated in patients with esophagitis [166]. Thus, exposure of the esophageal mucosa to gastric acid may also cause bronchospasm by activating these nociceptors and elicit vagal-mediated reflex and release of substance P in the bronchial mucosa [164].

Irritant Tracheobronchitis: Chronic exposure of the airways to chemical irritants, occupational and environmental pollutants often leads to development of irritant tracheobronchitis. For example, chronic exposure to airborne particulate matter, such as fly ash and smoke, is associated with respiratory disorders and increased morbidity. TRPV1 channels are expressed in cultured human airway epithelial cells, and particulate matter increases intracellular Ca^{2+} that was inhibited by capsazepine in 70% of the cells [167]. Particulate matter also induces epithelial apoptosis via an action on the TRPV1

receptor. Accordingly, inhibition of TRPV1 receptors has been suggested to be an effective pharmacological intervention to prevent certain pathological actions and toxicity induced by environmental particulate matters [3, 167]. In addition, prolonged exposure to inhaled irritants or air pollutants such as ozone, toluene diisocyanate or cigarette smoke has also been shown to enhance the sensitivity of airway sensory nerves to capsaicin as well as the synthesis of TKs and CGRP [11, 168, 169].

5. Current status on drug discovery

Several synthetic antagonists of the TRPV1 channel have been discovered and are under investigation mainly for alleviating the pain sensation. Several phase-II clinical trials on pain and migraine are currently in process. Airway diseases such as asthma and chronic cough have been listed as a potential indication for the TRPV1 antagonist drug discovery research. For example, Merck, in collaboration with Neurogen Corp, is developing an orally active TRPV1 antagonist, NGD-8243 (MK-2295), for the treatment of pain, urinary incontinence and asthma; a phase-II trial for pain began in November 2006. Glenmark Pharmaceutical Ltd also discovered a selective TRPV1 antagonist, GRC-6210, for the treatment of pain, urinary incontinence and asthma, and its phase-I clinical study began in October 2006. However, to our knowledge, no TRPV1 antagonist compound has yet been tested for treatment of airway diseases in humans.

6. Summary

Evidence obtained from electrophysiological and immunohistochemical studies has clearly demonstrated the expression of TRPV channels in various cell types in the

respiratory tract. However, the roles of these cation channels in regulating the overall respiratory function are still not clearly understood. TRPV1 is abundantly expressed in the unmyelinated sensory nerves innervating the airways and lung, and widely recognized as a transducer as well as an integrator of multiple physiological and environmental stimuli that the airways are exposed to. Recent studies began to uncover many of the endogenous substances that can either activate or regulate the sensitivity of TRPV1, which further characterize its important role in regulating the airway responsiveness, especially under pathophysiological conditions. These new findings further suggest the involvement of TRPV1 in the pathogenesis of certain airway diseases such as asthma and chronic cough, but definitive evidence has not yet been established. The investigation in search for answers to these questions is hindered by the unavailability of selective antagonists to the individual TRPV receptors, other than TRPV1. The development of TRPV-null mouse models have offered excellent potential to answer some of these important questions. More importantly, clinical studies designed to block selectively the TRPV channels in the airways of the patients with specific respiratory diseases are required to verify the role of TRPVs in humans. If their involvements are confirmed, TRPV channels could become important therapeutic targets for developing novel treatments of these respiratory diseases.

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Figure Legends :

Fig. 1. Role of TRPV1 in the acid-evoked currents in rat vagal pulmonary sensory neurons. Panel A: representative records illustrating the effects of amiloride (100 μ M; 2 min) and capsazepine (CPZ; 10 μ M, 2 min) on pH 5.5 (6 s)-evoked inward currents in a jugular neuron (23.1 pF). Panel B: group data showing the effects of 2-min pretreatment with amiloride (100 μ M) and CPZ (10 μ M) on both transient and sustained components evoked by low pH (6.5-5.5; 6 s). * $P < 0.05$ as compared with the corresponding control. Data represent means \pm SEM. (From Reference 44)

Fig. 2. Involvements of TRPV1 and other TRPVs in the responses of isolated rat vagal pulmonary sensory neurons to increase in temperature. Panel A: in current-clamp mode, increasing the temperature in a ramp pattern evoked depolarization and action potentials in a jugular neuron (21.0 pF). V_m , membrane potential. Insets display the action potential signals at an expanded time scale. Panel B: in voltage-clamp mode (holding potential = -70 mV), capsaicin (0.5 μ M, 2-s duration) and hyperthermia were applied to a jugular neuron (28.6 pF). Panel C: the consistent temperature-current relationships of the two different hyperthermia challenges in the same neuron (from data shown in Panel B).

$TI_{20\%}$ was determined by locating the temperature point where the amplitude of the current reached 20% of the peak current generated at 41°C. Panel D: representative traces illustrating the inward currents evoked by hyperthermia at control, after pretreatments with capsazepine (CPZ, 10 μ M), a selective TRPV1 antagonist, and ruthenium red (RR, 3 μ M), a non-selective but effective blocker of TRPV1-4 channels, and after washout in a jugular neuron (28.6 pF). At least 10 min was allowed for the cell to recover between tests. Panel E: the temperature-current relationships of the four current traces shown in

Panel D. Panel F: group data showing the effects of different treatments on cell response to hyperthermia. Data are means \pm SEM ($n = 11$; RR was studied in only 9 of these cells). *, significantly different ($P < 0.05$) from the control response; †, significant difference ($P < 0.05$) between the responses after CPZ and RR treatments. (Modified from reference 4)

Fig. 3. Immunofluorescence staining of airway nerves in a bronchial biopsy with an anti-TRPV1 antibody in a normal non-coughing volunteer (norm; Panel 1) and in a patient with chronic cough (cough; Panel 2). Panel 3 shows no staining with the negative control when the primary antibody was not added. Individual percentages of bronchial epithelial area stained positive for TRPV-1 was significantly greater in patients with chronic cough than in healthy volunteers ($P < 0.001$; Panel 4). Arrows denote positive neuronal staining; original magnification $\times 250$. There was a significant correlation ($r = -0.41$; $P < 0.05$) between TRPV-1 expression (percentage of epithelial area) and the capsaicin concentration PC_5 in the 29 patients with chronic cough (Panel 5); PC_5 is the concentration (μM) of capsaicin aerosol that evoked five coughs or more in individual patients. (Modified from reference 131)

Fig. 1

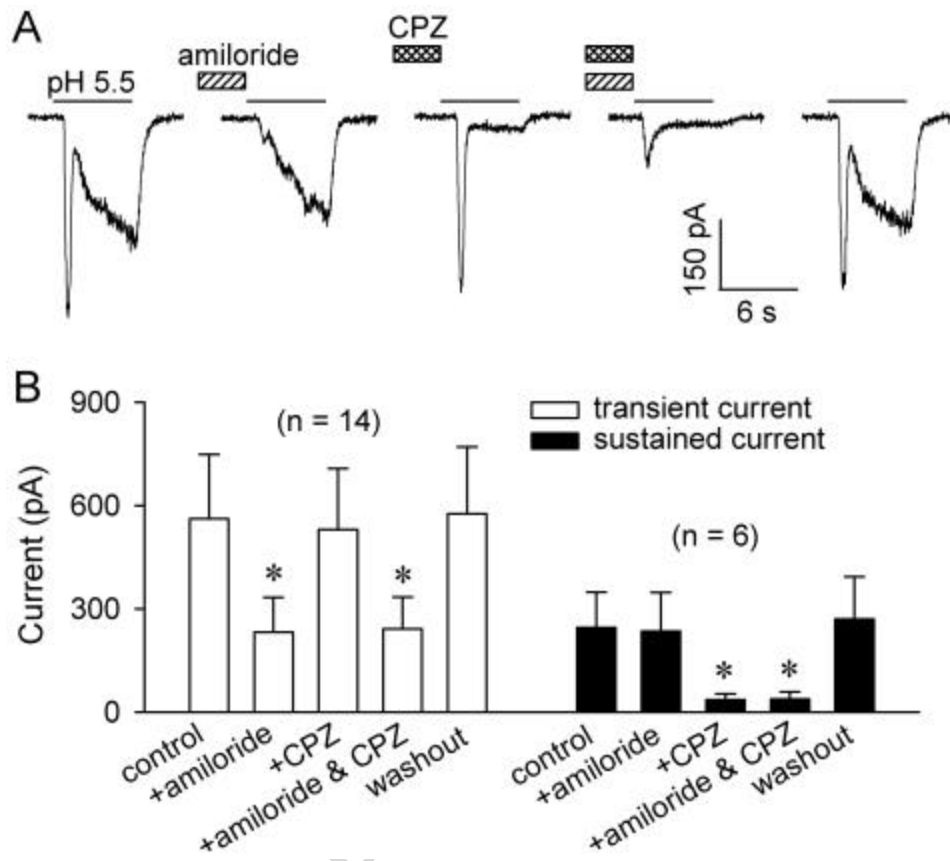


Fig. 2

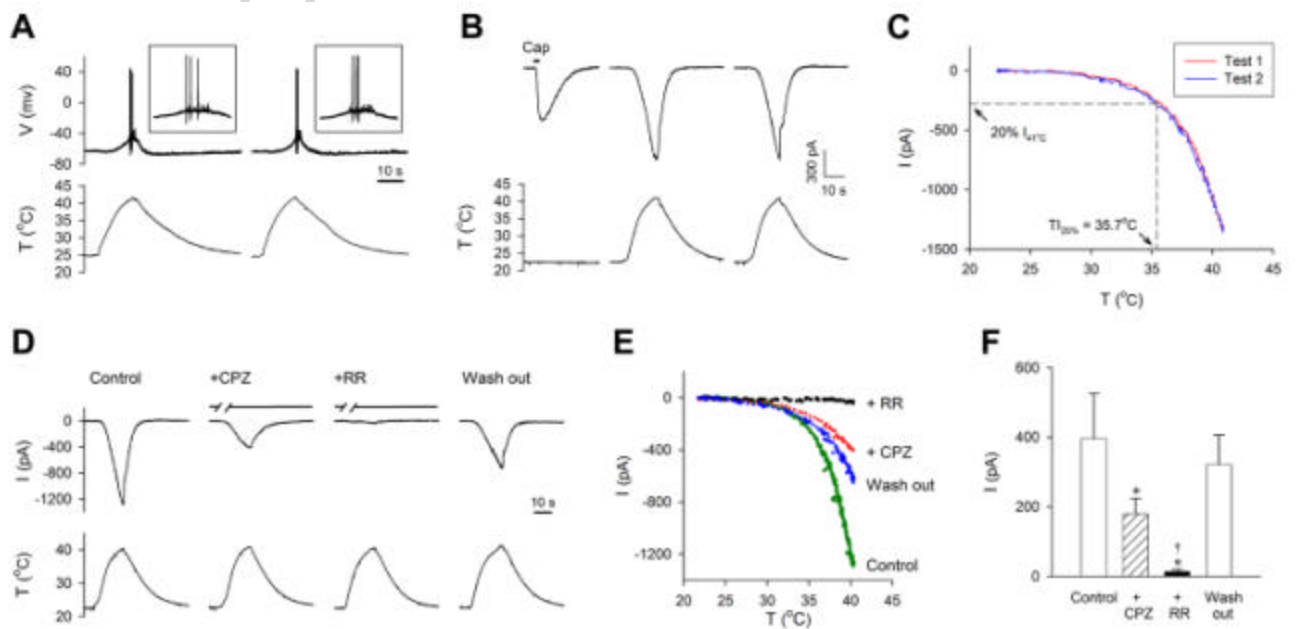


Fig. 3

