

Ion Channels in Asthma*

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Ion channels are specialized transmembrane proteins that permit the passive flow of ions following their electrochemical gradients. In the airways, ion channels participate in the production of epithelium-based hydroelectrolytic secretions and in the control of intracellular Ca^{2+} levels that will ultimately activate almost all lung cells, either resident or circulating. Thus, ion channels have been the center of many studies aiming to understand asthma pathophysiological mechanisms or to identify therapeutic targets for better control of the disease. In this minireview, we focus on molecular, genetic, and animal model studies associating ion channels with asthma.

Asthma is an inflammatory disorder of the conducting airways that is characterized by generalized reversible obstruction of the airflow and affects between 1 and 18% of the population depending on the country (1). Asthma etiology is complex and multifactorial in that both a hereditary component (one or more containing genetic variations that enhance susceptibility) and the environment participate (2, 3). Chronic inflammation is associated with bronchial hyper-responsiveness (BHR),² which leads to recurrent episodes of shortness of breath, coughing, and wheezing. At the pathophysiological level, asthma results from complex biological interactions between different cell types, both resident (*i.e.* epithelial and smooth muscle cells) and circulating (mainly immune cells), and environmental factors such as allergens, infections, and tobacco smoke (1, 4). A key element in this pathophysiological process is the T-helper 2 ($\text{T}_\text{H}2$) cell, which orchestrates chronic inflammation, smooth muscle contraction, and airway remodeling (3, 4). Another key feature is a defective airway epithelium, facilitating allergen contact with mucosal antigen-presenting dendritic cells (DCs),

which in turns will promote a $\text{T}_\text{H}2$ cell phenotype (5, 6). Other immune cells such as B lymphocytes, mast cells, and eosinophils as well as sensory neurons innervating the airway and endothelial cells involved in vascular permeation also participate (7–10).

Ion channels regulate many key functions of the cells implicated in asthma pathophysiology (Fig. 1). Therefore, intense research on the channel contribution to the genesis or therapy of the disease has been carried out over the last 30 years. Similar to asthma pathogenesis, which has moved from an intrinsic airway smooth muscle (ASM) abnormality to an autonomous nervous system dysfunction to the present-day inflammatory disorder, the role of ion channels in asthma has also evolved. The initial interest in ion channels was classically centered on their role in ASM contraction. Following the identification of voltage-gated Ca^{2+} channels (VGCCs) responsible for smooth and cardiac muscle contraction and their pharmacological inhibition in the 1970s (11), these channels were capitalized on in early asthma studies (12, 13). Interest then focused on the potassium channels that modify membrane potential and, consequently, the activation of VGCCs in smooth muscle (14, 15). Because of their crucial involvement in many airway epithelial functions and smooth muscle contraction (16–19), chloride channels have also appeared recurrently in asthma studies. Nowadays, the focus has moved away from ASM channels toward those involved in sensing irritants or the inflammatory response, particularly, the nonselective cationic transient receptor potential (TRP) channels (20, 21).

Additional support for the role of ion transport in the pathogenesis of asthma has recently and unexpectedly come from a genetic association study. A genome-wide association study of childhood asthma showed the strongest and almost exclusive association with the *ORMDL3* gene (22). The product of this gene is an endoplasmic reticulum (ER) protein that participates in ER-mediated Ca^{2+} homeostasis and stress responses (23). There are many channels in airway cells that have been analyzed, the function of which may contribute to the disorder, but because of the short format of this minireview, we focus primarily on those ion channels whose association with asthma pathogenesis or its clinical manifestations has been evaluated in molecular, genetic, or animal model studies.

Epithelial Ion Channels

Early observations carried out in asthmatic patients revealed the presence of a damaged epithelium (24), which may facilitate the permeability of the airways to inhaled irritants, allergens, and pathogens as well as the exposure of sensory nerves and the release of inflammatory mediators. Currently, it is postulated that allergen sensitization may well be the consequence of a defective airway epithelium (5, 6), leading to inappropriate programming of mucosal DCs (25, 26). An important factor that contributes to an impaired barrier function is the presence of defective epithelial tight junction formation or epithelial repair mechanisms. Both processes appear to be influenced by ion transport systems that may work independently of their trans-

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² The abbreviations used are: BHR, bronchial hyper-responsiveness; $\text{T}_\text{H}2$, T-helper 2; DC, dendritic cell; ASM, airway smooth muscle; VGCC, voltage-gated Ca^{2+} channel; TRP, transient receptor potential; ER, endoplasmic reticulum; CFTR, cystic fibrosis transmembrane conductance regulator; ENaC, epithelial Na^+ channel; CRAC, calcium release-activated current; SOCE, store-operated Ca^{2+} entry; KO, knock-out.

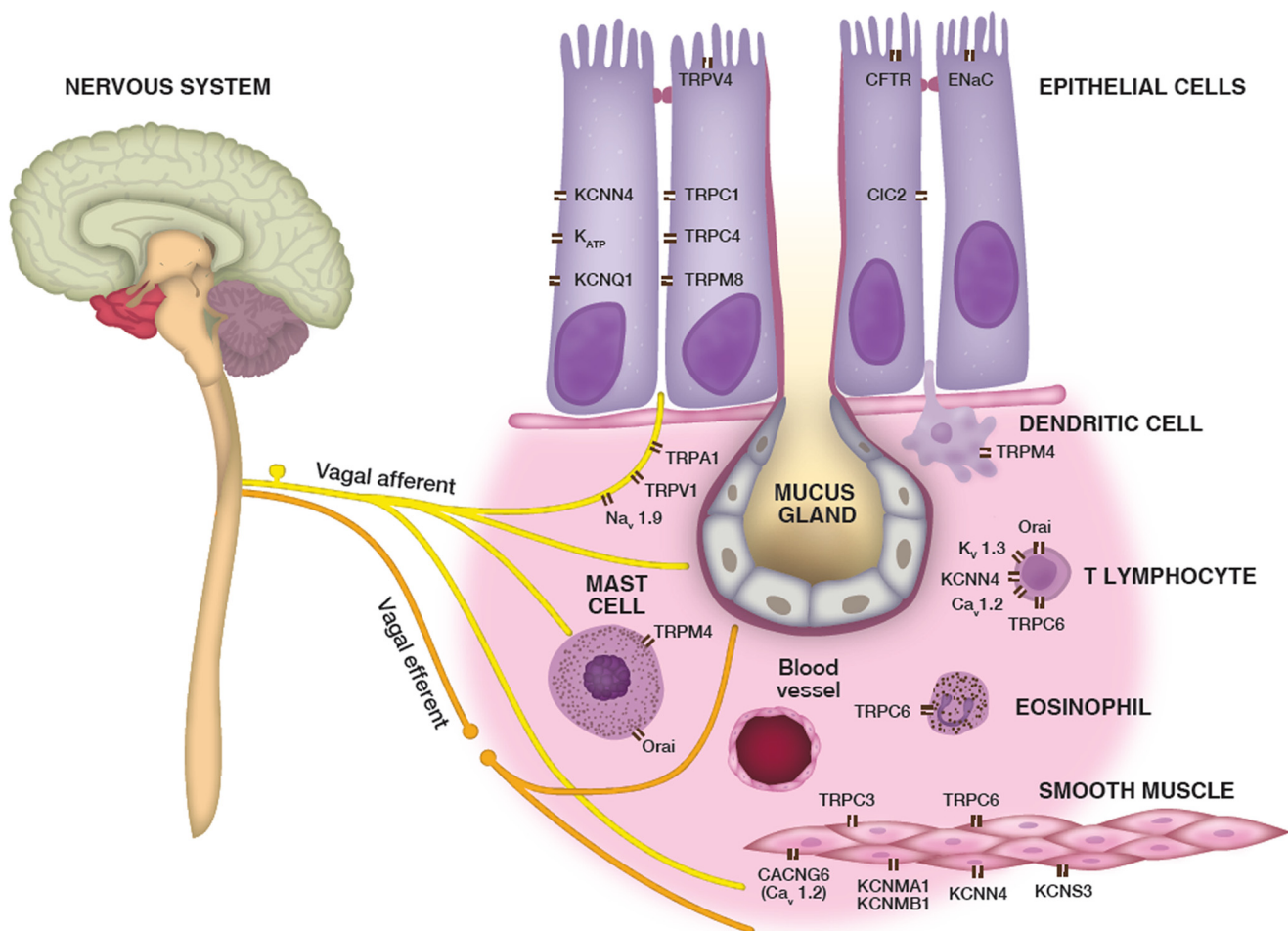


FIGURE 1. **Ion channels and asthma.** This schematic overview of the different airway cells shows the ion channels associated with asthma pathophysiology or its clinical symptoms. See text for a detailed explanation.

port function (27, 28). In the airways, several ion channels have been linked to tight junction formation, epithelial permeability, or repair: the cystic fibrosis transmembrane conductance regulator (CFTR) (29, 30) and the $K_v7.1$ (KCNN1), $Kir6.1$ (K_{ATP}), and $K_{Ca}3.1$ (KCNN4) potassium channels (31). Other channels that are also expressed in airway epithelia, although their roles in epithelial barrier or repair functions have been demonstrated elsewhere, include $CIC2$ (32), $TRPC1$ (33), $TRPV4$ (34), and $TRPC4$ (35). Considering that these ion channel-dependent cell processes are common denominators in asthma pathophysiology, their study (either measuring function or expression levels) in asthmatic airways or in animal models may provide novel insights into the pathogenesis of the disorder.

The sensory neuron $TRPV1$ channel (the founding member of the vanilloid subfamily of TRP channels (36)) has also been detected in immortalized human airway epithelial cell lines and implicated in particulate matter-induced apoptosis (37), thereby affecting the integrity of the epithelial barrier. However, no response to capsaicin, the classical $TRPV1$ activator, has been observed in native mouse tracheal epithelial cells (Fig. 2). It would be interesting to test whether the native human airway epithelium expresses functional $TRPV1$ channels. $TRPM8$, a member of the TRPM subfamily (melastatin) that

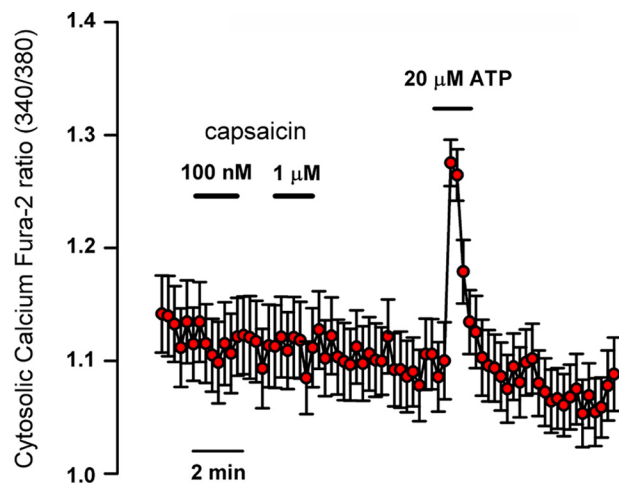


FIGURE 2. **Calcium responses to activators of $TRPV1$ and purinergic receptors in mouse tracheal ciliated cells.** Shown are average calcium increases measured with the Ca^{2+} sensor Fura-2 in a primary culture of mouse tracheal cells exposed to two different concentrations (100 nM and 1 μ M) of the $TRPV1$ activator capsaicin. Under these conditions, ciliated epithelial cells did not respond to capsaicin but did respond to ATP (20 μ M), a typical physiological activator of purinergic receptors. Results are expressed as the mean $\pm$ S.E. of 10 cells.

functions as a cold transducer in the somatosensory system (38, 39), mediates cold-dependent increased transcription of epithelial cytokine and chemokine genes (40) and may therefore

participate in the cold-induced aggravation of respiratory symptoms and asthma (41).

Other functions of conducting airway epithelia related to hydroelectrolytic transport, osmomechanical responses, and mucociliary clearance are also linked to the activity of ion channels and/or intracellular calcium signaling (16, 42–47). Of particular interest for airway pathophysiology are the CFTR Cl^- channel and the epithelial Na^+ channel (ENaC). Mutations in the CFTR gene result in cystic fibrosis, a disease characterized by altered Cl^- and Na^+ channel activities that result in airway mucus obstruction, infection, and inflammation (48). CFTR and ENaC channels participate in fluid secretion and reabsorption, thereby controlling the volume and composition of the airway surface liquid, which in turn affects ciliary beating and mucociliary clearance (49). Defects in airway cilia (structural or functional) affect the incidence of respiratory infection, but the presence of primary mucociliary dysfunction in asthmatics is still a matter of debate, probably being more relevant to chronic obstructive pulmonary disease (50). Transgenic β ENaC mouse models present many characteristics of the airway inflammatory response in the absence of pathogens (51), and reduced expression of all ENaC subunits has been found in preterm infants with respiratory distress (52). To date, there is no evidence for a direct association between ENaC or CFTR malfunctioning and asthma, apart for one study that associated several CFTR mutations with asthma, although those mutations were also found in healthy individuals, and subsequent studies did not support the original findings (53). Other airway epithelial channels have also been the subject of genetic epidemiological studies. A loss-of-function SNP (54) in the TRPV4 channel involved in ciliary beating frequency regulation (46, 55) has shown no association with asthma (56) but was associated with chronic obstructive pulmonary disease (57) and hyponatremia (54).

ASM Ion Channels

ASM controls airflow through the conducting airways. Its contraction reduces airflow, whereas relaxation facilitates it. ASM plays a central role in BHR and remodeling (58) and has been the subject of intense research to identify the molecular mechanisms participating in its contraction, proliferation, and migration. Ion channels facilitating ASM contraction aim to increase overall intracellular Ca^{2+} concentration (e.g. VGCCs (59)), whereas those favoring bronchodilatation generally produce the opposite effect (e.g. potassium channels (60)). The role of ion channels in ASM contraction and asthma pathophysiology has been critically reviewed (61, 62), and the initial emphasis on VGCC blockers and potassium channel openers has not been warranted by their success in clinical trials (Refs. 14 and 63 and references within).

ASM presents voltage-dependent L-type (Ca_v1) and T-type (Ca_v3) Ca^{2+} channels (59). Activation of L-type channels following membrane depolarization (and their interplay with ryanodine receptors in the ER) triggers an increase in $[\text{Ca}^{2+}]$ and ASM contraction. Interestingly, the regulatory γ -subunit of the L-type channel (CACNG6), which stabilizes the inactivation of the channel, has been recently associated with aspirin-intolerant asthma in a Korean population (64).

Potassium channels contribute to the relaxation of ASM by hyperpolarizing the membrane potential and thereby preventing the activation of VGCCs. Electrophysiological and molecular approaches have facilitated the identification of several K^+ channels in ASM (although for some, only indirect evidence exists): Ca^{2+} -activated K^+ channels (K_{Ca}), voltage-activated K^+ channels (K_v), and ATP-sensitive K^+ channels (K_{ATP}) (65–68). Despite their clear contribution to ASM physiology, evidence for their involvement in asthma pathophysiology is scant. Loss-of-function SNPs of the regulatory β_1 -subunit (KCNMB1) of the pore-forming α -subunit of the voltage- and Ca^{2+} -activated large conductance K^+ channel ($\text{K}_{\text{Ca}}1.1$, KCNMA1; also known as BK) have been associated with asthma severity in African Americans (69). However, a BK channel-deficient mouse model presented an unexpected reduced (rather than increased) ASM contractility due to a compensatory up-regulation of the cGMP pathway, which may reflect the important role of BK channels in ASM contraction (70). BK channel impact on ASM relaxation has received further support from a very recent study showing that bitter tastants activate BK channels and relax the airways of an asthma mouse model with higher efficacy than the currently used β -agonists (71). In addition to regulating ASM contraction, the $\text{K}_{\text{Ca}}3.1$ channel (also known as KCNN4 or IK_{Ca}) has also been implicated in ASM proliferation, being up-regulated by TGF- β , a regulatory process that is more pronounced in asthmatics (66). Pharmacological inhibition of $\text{K}_{\text{Ca}}3.1$ prevents proliferation of ASM (66, 72) and modulates the function of $\text{K}_{\text{Ca}}3.1$ -expressing immune cells (see below). Another ASM K^+ channel relevant to asthma pathophysiology is KCNS3, a non-conducting α -subunit $\text{K}_v9.3$ channel with a regulatory function in $\text{K}_v2.1$ (KCNB1) channels. Different SNPs in KCNS3 have been associated with airway hyper-responsiveness, although no functional dysregulation has been proven (73).

Several TRP channels have also been identified in ASM (20, 74), but only those contributing to BHR and/or remodeling are discussed. Most TRP channels are nonselective channels that mediate intracellular Ca^{2+} increases either directly or via membrane depolarization and activation of VGCCs. The TRPC1 channel contributes to ASM proliferation (75) and presumably airway thickening, whereas the main role of the TRPC3 and TRPC6 channels relates to ASM contraction (76, 77). In addition, TRPC3 expression in ASM increases in the ovalbumin-sensitized asthmatic mouse model (76) and in response to the proinflammatory cytokine TNF- α (78), which raises the question of whether the efficacy of TNF- α antagonists in the treatment of asthma (79) may also involve TRPC3.

Ion Channels in Immune Cells

As in many other cells, the main function of ion channels in immune cells is to control cytosolic Ca^{2+} signals, which in turn will regulate short-term (i.e. mast cell degranulation) and long-term (i.e. T cell proliferation and cytokine production) cellular responses (80). Particularly relevant is the Ca^{2+} entry mechanism (the calcium release-activated current (CRAC) (81)) triggered by the cross-linking of antigen receptors, activation of the phospholipase C/inositol trisphosphate pathway, and the subsequent depletion of ER Ca^{2+} stores. This event, termed store-

operated Ca^{2+} entry (SOCE), relies on two recently discovered elements: the ER Ca^{2+} sensor STIM, which communicates to the plasma membrane Ca^{2+} channel Orai the need to replenish the intracellular store (82). Considering the key role played by immune cells in asthma pathogenesis and that their activation is typically linked to SOCE mechanisms, it is surprising that only a few studies have focused on SOCE in the context of immune cell function in asthma. Blocking CRAC prevents $\text{T}_\text{H}2$ cell-mediated responses in a murine model of asthma (83), whereas mast cells derived from STIM1 knock-out (KO) and Orai1-KO mice present defective degranulation and activation of transcription factors NF-AT and NF- κ B (84, 85).

The function of many other ion channels in immune cells is principally to regulate CRAC by modulating the driving force for calcium entry through Orai channels. Potassium channel activation hyperpolarizes the cell membrane potential, thereby favoring Ca^{2+} entry via channels other than VGCCs, whereas K^+ channel inhibition prevents it. Both voltage-dependent ($\text{K}_\text{v}1.3$) and Ca^{2+} -dependent ($\text{K}_{\text{Ca}3.1}$) K^+ channels regulate T cell activation and proliferation (86, 87), and the latter have also been involved in mast cell IgE-mediated histamine release (88).

TRP channels are involved in different immune cell functions with relevance to asthma pathophysiology. TRPC6-KO mice show reduced airway eosinophilia, blood IgE levels, and $\text{T}_\text{H}2$ cytokines (IL-5 and IL-13), resulting in a decreased allergic airway response (77). The Ca^{2+} -activated nonselective cation channel TRPM4 contributes to membrane depolarization, thereby reducing SOCE due to a smaller Ca^{2+} -driving force after Fc ϵ receptor I stimulation of mast cells or chemokines in the case of DCs. Thus, TRPM4-KO mice show increased SOCE with a more severe IgE-mediated acute passive response (89) and altered migration of DCs (90).

To conclude, it is worth mentioning the unexpected but interesting role of $\text{Ca}_\text{v}1.2$ in $\text{T}_\text{H}2$ cytokine production and development of airway inflammation. $\text{Ca}_\text{v}1.2$ knockdown ameliorates the asthma induced in murine models (91).

Ion Channels in Sensory Nerves

Nerves innervating the lung control different aspects of the airway physiology: gland secretions, epithelial transport, dilation of vessels, and ASM contraction. Nerves also mediate different reflex responses, coughing and sneezing, aiming to protect the airways from chemical and biological challenges (92). The vagus nerve provides most of the nerves that innervate the airways (sensory and parasympathetic nerves), whereas sympathetic innervation comes from the spinal cord. Most important for asthma pathophysiology and several of its manifestations are the sensory nerves whose cell bodies are located in the nodose, jugular, and dorsal root ganglia. Abnormal neuronal function may contribute to airway disease. Stimulation of sensory terminals triggers protective reflex responses that, when occurring in the lower airways, may even produce bronchoconstriction and neurogenic inflammation by the release of inflammatory mediators. TRP channels are implicated in the detection and initiation of reflex responses to chemicals and are postulated to play a role in the pathogenesis of chronic respiratory diseases. TRPV1 activity has been related to neurogenic inflammation (93), irritant-induced chronic coughing (94), and

airway hypersensitivity (95). In addition, a loss-of-function mutation in TRPV1 is associated with a lower risk of presenting with wheezing and coughing in asthmatic children (56). Another TRP channel that has received considerable attention in recent times is TRPA1, as this channel appears to mediate the airway response to many different toxic gases and irritants, including cigarette smoke (96), nicotine (97), oxidants (98), heavy metals (99), and general anesthetics (100). TRPA1 activation evokes coughing in animal models and humans (101), and more impressively, TRPA1-KO mice show an alleviation of the inflammatory processes triggered by allergens in the ovalbumin model of asthma (102).

Conclusions

Asthma is a disorder presenting dysfunctional elements at all cellular levels in the airways, and ion channels regulate, one way or another, the function of all airway cells. The emphasis of ion channel research in asthma has been centered for a long time on ASM and immune cell channels, but it is now shifting toward the sensory channels of the nerves. Although ASM channel pharmacology has not been effective to date, the challenge now is to use the ion channels recently identified as key elements in asthma pathogenesis and responses to environmental factors as targets for the development of new pharmacological tools for novel and improved treatments.

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