BASIC SCIENCE FOR THE CHEST PHYSICIAN

Ion channels

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ABSTRACT

Effective therapies for the 10% of patients with 'severe' asthma remain elusive, while other pulmonary diseases such as idiopathic pulmonary fibrosis are currently untreatable. Many cellular processes rely heavily on signals delivered by changes in the intracellular-free Ca^{2+} concentration, in many cases relying on Ca^{2+} influx from the extracellular fluid through specific ion channels. This Ca^{2+} influx is, to some extent, dependent on the plasma membrane potential, which is controlled by the flow of other ions such as K^+ and Cl^- through their own channels. Irrespective of the point from where pathophysiological cell function is sustained, all mechanisms are predicted to rely heavily on the activity of the final effector ion channels required for pathological cell function. Ion channels are therefore highly attractive targets for the treatment of many diverse diseases, including those affecting the lung.

LUNG PATHOPHYSIOLOGY

There is a wealth of literature across the spectrum of pulmonary disease implicating various cytokines and receptor-dependent signalling pathways in disease processes. And while targeting these in artificial animal models is often effective, disappointingly few drugs reach man. The reasons for this are multifactorial, but include significant redundancy in cytokine networks and intracellular signalling pathways. Thus, in spite of billions of pounds spent on research, there have only been relatively modest advances in the treatment of many common pulmonary diseases over the last few decades.

ION CHANNELS

All cell processes rely heavily on the movement of monovalent and divalent ions. Since these are found in aqueous solution, cells use protein pores called ion channels to allow their passage in and out of the cell across the hydrophobic plasma membrane. These channels often consist of multiple subunits, each with several transmembrane domains, and have a central aqueous pore lined by channel-specific residues (figure 1A). A common structure among cation channels is a protein with six transmembrane domains with intracellular N- and C-termini, with the central pore generated by an intramembranous loop between the 5th and 6th transmembrane domains (figure 1A). The sequence -GYG- in this domain is highly selective for K^+. In simple terms, ion channels can be thought of as being closed, in which case ions cannot flow, or open in which case they can. Regulation of channel opening is called gating and can be controlled in a number of ways. Each gating stimulus leads to a conformational change in the channel, leading to pore 'opening'. For example, changes in plasma membrane potential acting on the voltage sensor in transmembrane segment 4, which contains several positively charged amino acids, lead to the opening of voltage-gated K^+ channels. Another gating mechanism is Ca^{2+} binding to calmodulin (CAM) attached to a CAM-binding site at the C terminus of Ca^{2+}-activated K^+ channels. Other channels are regulated by the binding of ligands such as ATP (ATP-dependent Kir 1.1 channels, P2X family of ATP receptor/channels) or cyclic nucleotides (cyclic nucleotide-gated channels), and most if not all channels can be further regulated by biochemical modifications such as phosphorylation. Store-operated CRACM/Orai Ca^{2+} channels are gated by the translocation of STIM1 from the endoplasmic reticulum Ca^{2+} stores to the plasma membrane following store depletion in response to cell stimulation and the generation of inositol-1,4,5-triphosphate (IP_3). The nomenclature for many channel families follows the format $Ion_{(gating\ mechanism)}$Family.Subfamily member, for example $K_v1.3$ is a voltage-gated K^+ channel belonging to family 1, member 3.

ION CHANNELS AS DRUG TARGETS

Cells such as muscle and nerves fire action potentials and are therefore known as excitable cells. The role of voltage-gated channels in propagating these electrical impulses is well described. In contrast, cells that do not generate action potentials are termed non-excitable cells. Non-excitable cells such as leukocytes, fibroblasts and epithelial cells also express a complex mix of ion channels carrying K^+, Cl^-, Ca^{2+} and non-selective combinations of cations. These channels are expressed to differing extents, depending on the cell type, its state of differentiation and level of activation. Influx of extracellular Ca^{2+} is an essential requirement for the activity of many cellular processes. However, K^+ channels play an important role in regulating Ca^{2+} signalling through their ability to maintain a negative membrane potential, and thus, allow CRACM/Orai Ca^{2+} channels to conduct larger currents during cellular activation. When K^+ channels open, K^+ moves out of cells due to the large chemical gradient (intracellular K^+ 140 mM, extracellular K^+ ~5 mM). This generates a negative or hyperpolarised cell membrane potential (figure 1B). Equilibrium is reached when negative intracellular charge counteracts the outward chemical driving force for K^+. For K^+ ions, this equilibrium sets the cell membrane potential to about −80 mV. As a result, open K^+ channels promote Ca^{2+} influx through CRACM/Orai channels in both T cells and mast cells, and both channel types accumulate at the immunological synapse[1 2] (figure 1C). There is therefore the potential to target Ca^{2+} channels in

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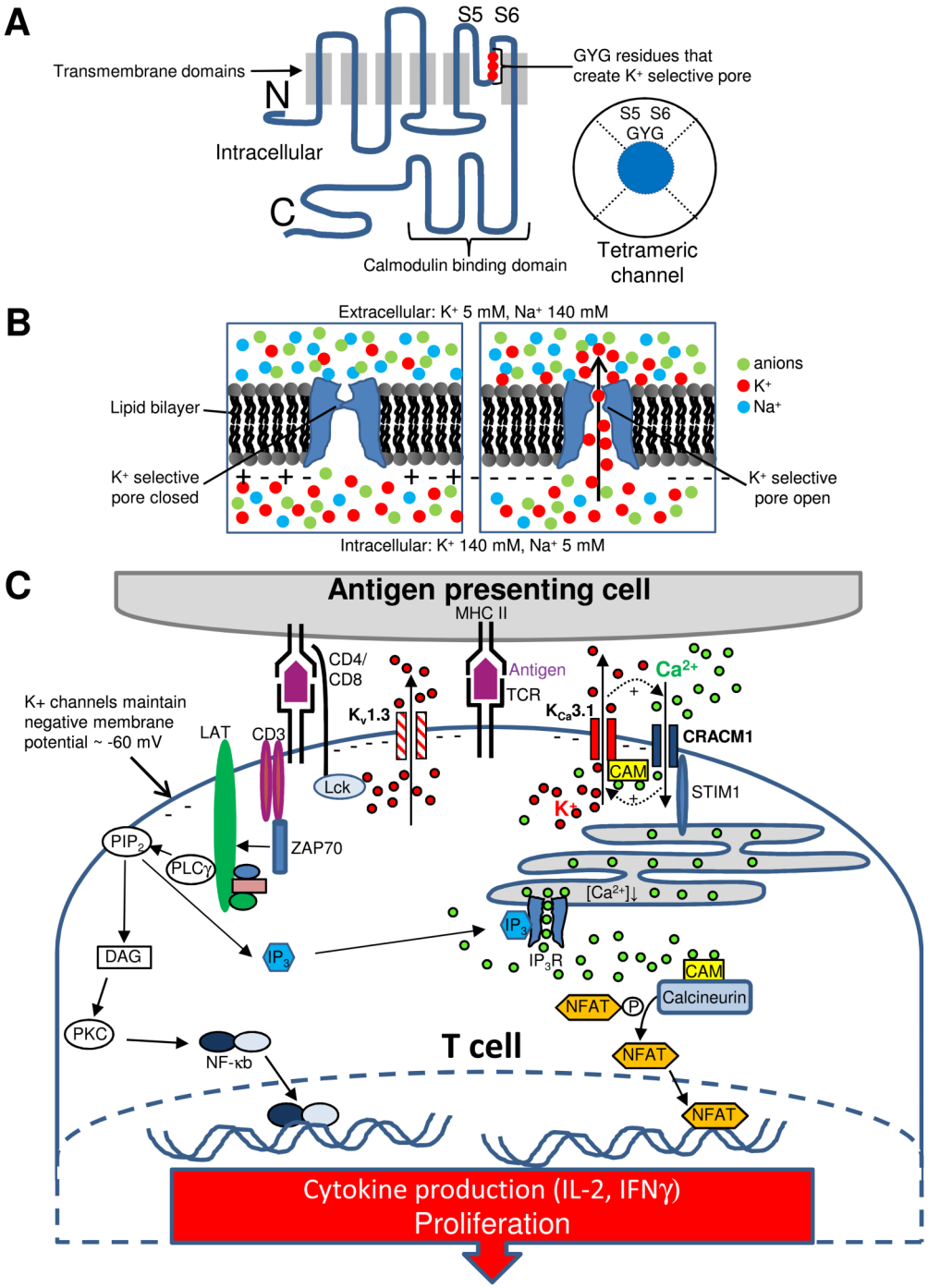

Figure 1 (A) Structure of the $K_{Ca}3.1$ ion channel. Four channel protein subunits assemble as a tetramer with apposition of the intramembranous -GYG- sequences creating the K^+-selective central pore. (B) Generation of a negative intracellular membrane potential following opening of K^+ channels. If all channels were closed, electrical charge across the cell membrane would be approximately 0 mV due the roughly equal distribution of cations and anions. If a K^+ selective channel opens, K^+ will move out of the cell down its concentration gradient, creating a negative charge intracellularly. This outward movement continues until the negative charge that accumulates is sufficient to counteract the further outward movement of K^+ ions. At this point, the ions are in equilibrium, and the cell membrane potential at this point is known as the equilibrium or reversal potential. For a K^+ channel, this potential is around −80 mV. (C) Involvement of $K_v1.3$, $K_{Ca}3.1$ and CRACM1 (Orail) at the immunological synapse between an antigen presenting cell such a dendritic cell or a macrophage and a T cell. Presentation of antigen leads to activation of the T cell receptor complex and the downstream activation of phospholipase Cγ (PLCγ) via the tyrosine kinases LCK and ZAP70. PLCγ catalyses the hydrolysis of the membrane phospholipid PIP_2 to inositol-1,4,5-triphosphate (IP_3) and diacylglycerol. IP_3 opens the IP_3 receptor (IP_3R) in the membrane of the endoplasmatic reticulum, resulting in the release of Ca^{2+} from intracellular stores. The rise in intracellular Ca^{2+} activates the phosphatase calcineurin, which then dephosphorylates the transcription factor nuclear factor of activated T cells, enabling it to translocate to the nucleus and to bind to the promoter of cytokine genes such as interleukin 2 (IL-2). CRACM/Orai, $K_v1.3$ and $K_{Ca}3.1$ channels cluster at the point of contact to critically regulate Ca^{2+} signalling. Depletion of internal Ca^{2+} stores induces the redistribution of STIM1 into sites adjacent to the plasma membrane, leading to activation of CRACM/Orai channels. The ensuing Ca^{2+} influx through CRACM/Orai channels depolarises the T cell (ie, makes the cell membrane more positive) and thus reduces Ca^{2+} entry through these channels, which conduct larger currents at negative membrane potentials. The driving force for Ca^{2+} entry is restored by membrane hyperpolarisation brought about by the opening of $K_v1.3$ channels in response

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non-excitable cells directly with CRACM/Orai channel blockers, or indirectly through the use of K^+ channel blockers.

EXAMPLES OF POTENTIAL ION CHANNEL TARGETS IN THE LUNG

There are many classes of ion channels expressed in pulmonary tissues and it is not possible to discuss them all in this short review. We will therefore give a few pertinent examples of ion channels with potentially important roles in pulmonary disease. We will not discuss the cystic fibrosis transmembrane regulator, which is a chloride transporter rather than channel, and which is discussed in detail in other texts.

$K_{CA}3.1$

Intermediate-conductance Ca^{2+}-activated $K_{Ca}3.1$ K^+ channels are opened by a rise in cytosolic free Ca^{2+} ($[Ca^{2+}]_i$) due to Ca^{2+}-CAM-mediated cross-linking of subunits in the channel tetramer. $K_{Ca}3.1$ channels are widely expressed in the human lung and are found in airway epithelial cells, fibroblasts, airway smooth muscle cells, endothelial cells, mast cells, macrophages and T cells. They play key roles in the migration of human lung mast cells (HLMCs) and fibrocytes (fibroblast progenitors), and their activation potentiates immunological HLMC and peripheral blood T cell activation. In contrast, in airway smooth muscle cells, their primary role appears to be control of cellular proliferation. Further work published in abstract form describes important roles for these channels in the regulation of parenchymal lung myofibroblast collagen secretion, contraction and wound healing. Taken together, these cellular studies suggest that targeting $K_{Ca}3.1$ channels may reduce airway inflammation, tissue remodelling and fibrosis.

This is supported by several in vivo studies. The $K_{Ca}3.1$ knockout mouse displays an attenuated IgE-dependent systemic anaphylactic response. In a mouse model of asthma, treatment with the specific $K_{Ca}3.1$ blocker TRAM-34 attenuated OVA-dependent bronchial hyperresponsiveness, bronchoalveolar lavage eosinophilia, sub-basement membrane collagen deposition, airway smooth muscle mass and peribronchiolar fibrosis.[3] Another mouse study demonstrated a role for $K_{Ca}3.1$ channels in the development of obliterative bronchiolitis following transplantation. These findings are in keeping with the abilities of $K_{Ca}3.1$ inhibition to attenuate renal fibrosis following ureteric obstruction, and to reduce atherosclerosis development in $ApoE^{-/-}$ mice by inhibiting both vascular smooth muscle cell proliferation and T cell and macrophage activity. These studies suggest that $K_{Ca}3.1$ inhibition may be useful for the prevention of lung remodelling and fibrosis, and may therefore, slow the progression of idiopathic pulmonary fibrosis.

CRACM/ORAI CHANNELS

Like $K_{Ca}3.1$, CRACM/Orai1-3 channels are widely expressed. They are central to many cell processes, providing a highly selective pathway for the entry of Ca^{2+} from the extracellular fluid following receptor-dependent depletion of Ca^{2+} from intracellular stores. In T cells (figure 1C), $K_{Ca}3.1$ and the voltage-gated $K_v1.3$ channel regulate Ca^{2+} influx through CRACM/Orai channels. This Ca^{2+} influx results in the increase in cytosolic Ca^{2+} concentration necessary for the translocation of the transcription factor nuclear factor of activated T cells (NFAT) to the nucleus and the initiation of new transcription ultimately resulting in cytokine secretion and T cell proliferation (figure 1C). Targeting CRACM/Orai channels therefore has the potential to ameliorate unhelpful T cell responses, such as those driving sarcoidosis.

CRACM/Orai channels also play a major role in the IgE-dependent activation of mast cells by allergen. Blocking or knocking out CRACM/Orai channels markedly reduces IgE-dependent mouse and HLMC degranulation, arachidonic acid metabolism and subsequent LTC_4 and PGD_2 production, and the release of several cytokines including interleukin 5 (IL-5) and IL-13.[4] In ex vivo human bronchi, CRACM/Orai blockade reduces the mast-cell-dependent contractile response of the airway to allergen. It is therefore likely that CRACM channel inhibition will inhibit the early and late airway responses following allergen challenge in vivo, and may in turn prove useful for the treatment of asthma.

TRANSIENT RECEPTOR POTENTIAL CHANNELS

The transient receptor potential (TRP) family of ion channels contains 28 mammalian members, which are subdivided into six main subfamilies based on sequence homology: TRPC (canonical), TRPV (vanilloid), TRPM (melastin), TRPP (polycystin), TRPML (mucolipin) and TRPA (ankyrin). The channels function predominantly as non-selective cation channels, most of which are permeable to Ca^{2+}. The selectivity of TRP channels for Ca^{2+} however varies greatly both across and within subfamilies.

TRP channels are widely expressed in both excitable and non-excitable cells, and have drawn particular interest in the lung with respect to the mechanisms of chronic cough. Both TRPA1 and TRPV1 are expressed in airway sensory nerves and implicated in the afferent sensory loop of the cough reflex. Both are activated by noxious stimuli, and implicated in both the initiation of cough reflexes, and sensitisation of the cough reflex by inflammatory mediators such as PGE_2 and bradykinin.[5] TRPV1 may also demonstrate increased expression in the airways of patients with chronic cough. Current TRPV1 inhibitors have potential problems due to the development of hyperthermia in guinea pig models and phase I trials of pain in humans, but topical delivery to the airway would potentially prevent this problem. Selective TRPA1 inhibitors are not currently available.

SUMMARY

In summary, ion-channel blockers currently treat a number of diseases including hypertension, angina, type-2 diabetes, cardiac arrhythmias and epilepsy. There is well-founded optimism that modulation of ion channels in excitable and non-excitable cells within the lung has the potential to attenuate many disease processes such as tissue fibrosis or symptoms such as chronic cough.

Contributors PB and HW wrote the manuscript.

Competing interests PB has acted as an advisor to Icagen, Inc. (2007–2010) and collaborated recently with GlaxoSmithKline on a CRACM ion channel project in mast cells. HW consulted for NeuroSearch A/S on $K_{Ca}3.1$ from 2009 to 2011.

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